Universidade de Aveiro Departamento de Biologia
2016

Sónia Dias Coelho

Vias de exposição humana a contaminantes ambientais

Human exposure pathways to environmental contaminants

Universidade de Aveiro Departamento de Biologia
2016

Sónia Dias Coelho

Vias de exposição humana a contaminantes ambientais

Human exposure pathways to environmental contaminants

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor António José Arsénia Nogueira, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro, da Doutora Ana Catarina Almeida Sousa, Estagiária de Pós-Doutoramento do CICECO, Departamento de Química da Universidade de Aveiro e do Doutor Shinsuke Tanabe, Professor Catedrático da Universidade de Ehime

Apoio financeiro do POCTI no âmbito do III Quadro Comunitário de Apoio. Cofinanciamento do POPH/FSE

Apoio financeiro da FCT no âmbito do III Quadro Comunitário de Apoio através da Bolsa de Investigação SFRH/BD/78168/2011

o júri

presidente	Prof. Doutor Valeri Skliaro professor catedrático da Universidade de Aveiro
vogal	Prof. Doutora Lúcia Maria das Candeias Guilhermino professora catedrática do Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto
vogal	Prof. Doutor Amadeu Mortágua Velho da Maia Soares professor catedrático do Departamento de Biologia da Universidade de Aveiro
vogal	Prof. Doutor António José Arsénia Nogueira (orientador) professor associado com agregação do Departamento de Biologia da Universidade de Aveiro
vogal	Prof. Doutor Luís Taborda – Barata professor associado com agregação da Faculdade de Ciências da Saúde da Universidade da Beira Interior
vogal	Doutor Tomohiko Isobe investigador sénior do *Center for Health and Environment Risk Research, National Institute for Environmental Studies*, Japão
vogal	Doutor João Paulo Fernandes Teixeira investigador auxiliar do Instituto Nacional de Saúde Dr. Ricardo Jorge, Porto
vogal	Doutora Ana Catarina Almeida Sousa (co-orientadora) bolseira de pós-doutoramento do CICECO, Departamento de Química da Universidade de Aveiro

agradecimentos

My PhD journey allowed me to work and learn from the best. These people passed me their knowledge without any restrictions, and I will be forever grateful. My deepest thanks go to:

My supervisor, Professor António J.A. Nogueira, which immediately accepted this position. Thanks for being my supervisor for the second time and for, once again, being available whenever needed.

My co-supervisor, Dr. Ana C.A. Sousa, which made this project possible. Thanks for teaching me how to be a good researcher, how to face the difficulties, for guiding me, for every effort over these 5 years and for trusting in my work and capacities.

My co-supervisor, Professor Shinsuke Tanabe, thanks for receiving me in his laboratory and for treating me as a member of the CMES, and for giving me the opportunity to learn all about the global environmental concern, it completely changed my vision as a scientist. I also thank for having provided me the best professional experience of my life.

Dr. Tomohiko Isobe, for receiving me as his student and share his knowledge. Thanks also for being the best host ever.

Dr. Ramiro Pastorinho, Dr. Tatsuya Kunisue and Dr. Takaaki Itai, for all the scientific opinions and discussions.

My lab mates, Dr. Nguyen Minh Tue, Nishioka-san, Okubo-san and Adi-san for teaching me the lab rules, the techniques and for the scientific discussions. Thanks mainly for being so patient and for being always available to help. I would not have come this far without you.

Thanks to all my colleagues/friends at CMES, for making me feel home, you're the best! Thanks, Mari-san, Rumi-san, Hazuki-san, Rina-san, Imai-san, Nomiyama-san, Kurata-san, Yamamoto-san, Nagano-san, Matsuda-san, Kana-san, Aki-san, Bom-san, Kei-san…

From now on I will thank in Portuguese:

Obrigada ao Dr. Luís Taborda-Barata, por me ajudar, através do seu ficheiro de utentes, na recolha de amostras e pela disponibilidade.

Agradeço a todas as pessoas que se voluntariaram para participar na recolha de amostras. Obrigada porque perceberam a importância deste trabalho e pelo esforço em colaborar connosco.

agradecimentos (cont.)

À Caty, desta vez na categoria de amiga e confidente. Tu estiveste sempre lá, e quando digo sempre, é sempre mesmo! Sem ti nada disto seria possível, por mil e uma razões. E claro, ao Ramiro, na categoria de amigo, por me ajudares direta e indiretamente e por me fazeres olhar sempre um pouco mais além.

Su, Ana, Belucha e Pituxa, por serem os meus companheiros cientistas. Obrigada por todas as discussões científicas e menos científicas. Convosco, ser "investigador" é fácil. Pituxa, padrinho, companheiro, confidente… Obrigada!

Á Raquel porque me deste a oportunidade de, melhor ou pior, ter a experiência de como também posso transmitir o meu conhecimento.

Em último e não menos importante, as pessoas que me enchem o coração:

Ao Tiago, por seres a melhor escolha de sempre, o meu Amigo e a minha força. Contigo é tudo mais simples!

Aos meus pais, por serem as minhas pessoas, por me apoiarem incondicionalmente e por terem tanto orgulho nas minhas conquistas sem saberem bem de quê que se trata.

À Bárbara e ao Jorge, por serem o meu melhor exemplo, por me completarem e me darem força. À Matilde, porque mesmo sem saber, ajuda-me tanto! Vocês são tudo…

À minha avó por estar sempre presente, mesmo estando longe. O teu orgulho também chega ao Norte.

Aos meus amigos, Sofia, Paulo e a minha Luisinha, Clã, Cecília, Bruno e 4º esquerdo por vibrarem com o meu sucesso tanto quanto eu. Obrigada!

Aos meus colegas/amigos do "outro" laboratório pela companhia, boa disposição e ajuda.

E a todas as outras pessoas, que não nomeei, mas que me acompanharam ao longo deste percurso, o meu mais sincero obrigada!

palavras-chave

Alimentação; bifenilos policlorados; cádmio; chumbo; metais tóxicos; pesticidas organoclorados; pó doméstico; poluentes orgânicos persistentes; Portugal; retardantes de chama.

resumo

Os humanos estão permanentemente expostos a vários contaminantes ambientais que têm sido produzidos pela indústria química ao longo das últimas décadas. Para além do contacto direto com produtos onde estes contaminantes são aplicados, a exposição ocorre principalmente porque estes químicos se libertam destes materiais e acumulam-se no ambiente, tornando-se desta forma disponíveis para serem consumidos involuntariamente pelos humanos. Durante muito tempo a alimentação foi considerada como a principal via da exposição dos humanos a diversas classes de contaminantes, incluindo os poluentes orgânicos persistentes (POPs) e metais tóxicos. No entanto, a exposição através do pó em ambientes interiores surgiu como uma importante via de exposição, principalmente porque estes contaminantes se acumulam no pó e devido ao facto das pessoas passarem grande parte do seu tempo em ambientes interiores.

Na presente tese, foi estudada a presença de várias classes de contaminantes ambientais em amostras de duplicados de dieta e amostras de pó doméstico, de forma a caracterizar a exposição dos humanos através da ingestão de alimentos e pó doméstico em Portugal, e a avaliar os riscos associados a esta exposição. Os contaminantes estudados incluem: retardantes de chama bromados (BFRs); retardantes de chama fosforados (PFRs); bifenilos policlorados (PCBs); pesticidas organoclorados (OCs); e os metais tóxicos chumbo (Pb) e cádmio (Cd).

Os níveis de BFRs, PCBs, OCs, Pb e Cd foram determinados em amostras de duplicados de dieta fornecidas por voluntários da comunidade académica da Universidade de Aveiro. No que diz respeito aos compostos orgânicos, as concentrações obtidas foram baixas. Os BFRs foram detetados em poucas amostras de dieta, sendo que o mais detetado foi o congénere BDE 209 (67%), enquanto os BFRs emergentes – 1,2-bis (2,4,6-tribromofenoxi) etano (BTBPE), decabromodifenill etano (DBDPE) – não foram detetados. Os PCBs e os OCs apresentaram as concentrações mais elevadas e foram detetados na maioria das amostras de duplicados de dieta analisadas, sendo que os diclorodifeniltricloroetanos (DDTs) e os hexaclorociclohexanos (HCHs) foram detetados em 100% das amostras. Os valores estimados das ingestões diárias foram baixos e abaixo dos valores de referência estabelecidos para a avaliação de risco em humanos.

O Pb foi detetado em todas as amostras de duplicado de dieta e o seu consumo através da ingestão de alimentos foi associado a efeitos adversos para a saúde.

resumo (cont.)

Para 33% dos participantes a ingestão diária estimada (EDI) foi superior à dose de referência (*bench mark dose level* – BMDL) associada à doença renal crónica, e para um dos participantes a EDI foi 50% mais elevado do que a BMDL associada à pressão arterial sistólica elevada. A abordagem da margem de exposição (MOE - *margin of exposure*) foi aplicada e indicou que em pelo menos 3,3 e 26,7% dos participantes poderão surgir efeitos cardiovasculares e nefrotóxicos, respetivamente. As concentrações de Cd foram avaliadas em amostras de duplicados de dieta fornecidas por mulheres a trabalhar ou a estudar na Universidade de Aveiro. Este metal foi também detetado em todas as amostras analisadas e 35% das participantes apresentaram ingestões semanais estimadas (EWIs) mais elevadas do que a dose semanal tolerável estabelecida, sugerindo riscos de saúde elevados. No geral, os resultados obtidos através das análises feitas em amostra de duplicados de dieta demonstraram que a ingestão de alimentos é uma importante via de exposição aos contaminantes ambientais estudados.

Os níveis de PFRs, BFRs and PCBs foram monitorizados em amostras de pó doméstico de casas de Aveiro e Coimbra. Estes compostos e os seus respetivos congéneres/isómeros foram detetados num grande número de amostras, sendo que os PFRs apresentaram as concentrações mais elevadas, seguidos dos BFRs e PCBs. Apesar das frequências de deteção elevadas, os EDIs foram inferiores às doses de referência (RfDs) estabelecidas. Os BFRs, PCBs, OCs foram também analisados em amostras de pó doméstico da Covilhã. Neste estudo, as amostras de pó foram recolhidas em casas de voluntários com asma e em casa de participantes sem asma. Os congéneres/isómeros dos contaminantes avaliados foram detetados na maioria das amostras, e o grupo de BFRs apresentou as concentrações mais altas, seguido de PCBs e DDTs. No entanto, para todos os contaminantes, as ingestões diárias foram inferiores às RfDs. Estes resultados confirmam que os PFRs, BFRs, PCBs e OCs estão omnipresentes nas casas Portuguesas, no entanto, o consumo diário dos contaminantes orgânicos através da ingestão de pó doméstico é baixo.

Os resultados obtidos no âmbito desta tese permitiram descrever, pela primeira vez em Portugal, os níveis dos contaminantes selecionados em amostras de duplicados de dieta e pó doméstico. Estes resultados revelaram que o risco associado à ingestão de contaminantes orgânicos através da alimentação e do pó doméstico foram baixos, ao contrário do elevado risco associado à ingestão de Pb e Cd presentes nos alimentos.

keywords

Cadmium; diet; flame retardants; house dust; lead; organochlorine pesticides; persistent organic pollutants; polychlorinated biphenyls; Portugal; toxic metals.

abstract

Humans are permanently exposed to environmental contaminants which have been produced for decades and with numerous applications. Besides the direct contact with the consumer products in which these contaminants are applied, the exposure occurs mainly because these chemicals are released from those materials and accumulate in the environment being available for involuntary consumption. For a long time, diet has been considered the major human exposure route for several contaminants, including persistent organic pollutants (POPs) and toxic metals. However, the indoor exposure through dust emerged as important exposure route, mainly motivated by the fact that these contaminants accumulate in dust and because people in modern society spend much of their time indoors.

In this thesis, several classes of environmental contaminants were analysed in duplicate diet samples and house dust in order to characterize the human exposure through the ingestion of food and the ingestion of house dust in Portugal and to access the associated risks. The contaminants studied include: brominated flame retardants (BFRs), phosphorus flame retardants (PFRs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCs), and also the toxic metals lead (Pb) and cadmium (Cd).

The levels of BFRs, PCBs, OCs, Pb and Cd were assessed in duplicate diet samples obtained from volunteers from the University of Aveiro community. Regarding the organic compounds the obtained levels were low. BFRs were detected in few duplicate diet samples, with the congener BDE 209 exhibiting the higher detection frequency (67%) while the emerging BFRs – 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE) – were not detected. PCBs and OCs exhibited higher levels and were detected in most analysed duplicate diet samples, with dichlorodiphenyltrichloroethanes (DDTs) and hexachlorocyclohexanes (HCHs) being detected in 100% of the samples. The daily dietary intakes were estimated, being low and under the established guidance values for human risk assessment. Pb was detected in all duplicate diet samples and its dietary ingestion was associated with adverse health effects. For 33% of the participants, the estimated daily intakes (EDIs) were higher than the dietary intakes related to the bench mark dose level (BMDL) derived from chronic kidney disease, and for one participant the EDI was 50% higher than the BMDL derived from elevated systolic blood pressure.

abstract (cont.)

The margin of exposure approach (MOE) was applied and indicated that cardiovascular and nephrotoxic effects might likely occur in at least 3.3 and 26.7% of the participants, respectively. The concentrations of Cd were assessed in duplicate diet samples provided by women working or studying in University of Aveiro. This metal was also detected in all analysed samples and 35% of the participants exhibited estimated weekly intakes (EWIs) higher than the established tolerable weekly intake (TWI), suggesting increased health risks. Overall the results from the duplicate diet study demonstrate that the ingestion of food is an important pathway of exposure to these environmental contaminants.

PFRs, BFRs and PCBs were monitored in house dust samples from two cities in central Portugal (Aveiro and Coimbra). These compounds and respective congeners/isomers were detected in a large number of samples, with PFRs exhibiting the highest concentrations followed by BFRs and PCBs. Despite their high detection frequencies, the EDIs were much lower than the established reference doses (RfDs). BFRs, PCBs and OCs were also analysed in house dust samples from Covilhã, Portugal. In this study, dust samples were collected from the houses of asthmatics and non-asthmatics participants. The contaminants congeners/isomers were detected in the majority of the dust samples, and the group of BFRs exhibited the higher concentrations, followed by PCBs and DDTs, however, the daily intakes were lower than the RfDs for all contaminants. These results confirm that PFRs, BFRs, PCBs and OCs are ubiquitously present in Portuguese households, however the daily intakes of these organic contaminants through house dust ingestion is low.

The results obtained under the framework of this thesis allowed describing for the first time in Portugal the levels of the selected contaminants in duplicate diet samples and in house dust samples. The results disclosed that the risk associated with the ingestion of the organic contaminants through diet and house dust was low, which contrasts with the risk associated with the ingestion of Pb and Cd through diet.

"Se podes olhar, vê. Se podes ver, repara."

José Saramago

List of Contents

Acronym List

Chemicals

α-HCH: alpha Hexachlorocyclohexane
β-HCH: beta Hexachlorocyclohexane
BFRs: Brominated Flame Retardants
BPA: Bisphenol A
BTBPE: 1,2-bis(2,4,6-tribromophenoxy) ethane
CHLs: Chlordanes
CECs: Contaminants of Emerging Concern
DBDPE: Decabromodiphenyl Ethane
DDT: Dichlorodiphenyltrichloroethane
dl-PCBs: dioxin-like Polychlorinated Biphenyls
DOP: Dioctyl Phthalate
EBFRs: Emerging Brominated Flame Retardants
EDCs: Endocrine Disrupting Chemicals
EHDPP: 2-Ethylhexyl Diphenyl Phosphate
FRs: Flame Retardants
HBCDD or HBCD: Hexabromocyclododecane
HCB: Hexachlorobenzene
HCHs: Hexachlorocyclohexanes
MeOH: Methanol
NBFRs: Novel Brominated Flame Retardants
ndl-PCBs: nondioxin-like Polychlorinated Biphenyls
OCs: Organochlorine Pesticides
PAHs: Polycyclic Aromatic Hydrocarbons
PBBs: Polybrominated Biphenyls
PBDEs: Polybrominated Diphenyl Ethers
PCBs: Polychlorinated Biphenyls
PCDDs: Polychlorinated Dibenzo-p-Dioxins
PCDFs: Polychlorinated Dibenzofurans
PFCs: Perfluorochemicals
PFOA: Perfluorooctanoic Acid
PFOS: Perfluorooctane Sulfonate
PFRs: Phosphorus Flame Retardants
POPs: Persistent Organic Pollutants
SVOC: Semi-Volatile Organic Compound
TBBPA: Tetrabromobisphenol-A
TBECH: 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane
TCEP: Tris(2-chloroethyl) Phosphate
TDCIPP: Tris(1,3-dichloro-2- propyl) Phosphate
TEHP: Tris(2-ethylhexyl) Phosphate
TMPP: Tricresyl Phosphate
TNBP: Tri-n-butyl Phosphate
TPEP: Tripentyl Phosphate
TPHP: Triphenyl Phosphate
TPP: Tripropyl Phosphate
tris-BP: Tris(2,3- dibromopropyl) Phosphate

Risk assessment

BMDL: Bench Mark Dose Level
CKD: Chronic Kidney Disease
DALYs: Disability-Adjusted Life Years
EDI: Estimated Daily Intake
EWI: Estimated Weekly Intake
DN: Developmental Neurotoxicity
IEUBK: Integrated Exposure Uptake Biokinetic
MOE: Margin of Exposure Approach
PTWI: Provisional Tolerable Weekly Intake
RfD: Reference Dose
SBP: Systolic Blood Pressure
TEQ: Toxic Equivalent
TDI: Tolerable Daily Intake
TWI: Tolerable Weekly Intake

Others

BMI: Body Mass Index
DDS: Duplicate Diet Study
dw: dry weight
EU: European Union
FFQ: Food Frequency Questionnaire
GC: Gas Chromatograph
GPC: Gel Permeation Chromatography
ICP-MS: Inductively Coupled Plasma Mass Spectrometer
IQ: Intelligence Quotient
IS: Internal Standards
LB: Lower Bound
LOD: Limit of Detection
lw: lipid weight
MBS: Market Basket Study
MDL: Method Detection Limit
MS: Mass Spectrometer
QA: Quality Assurance
QC: Quality Control
TDS: Total Diet Study
UK: United Kingdom
UP: Upper Bound
UPLC: Ultra-Performance Liquid Chromatograph
US: United States
ww: wet weight

Organizations

ADRA: Municipal Water Distribution System for the Aveiro Region
ATSDR: Agency for Toxic Substances and Disease Registry
BSEF: Bromine Science Environmental Forum
CONTAM: Panel on Contaminants in the Food Chain
EEC: European Economic Community
EFSA: European Food Safety Authority
FAO: Food and Agriculture Organization
IARC: International Agency for Research on Cancer
INE: National Institute of Statistics
IPCS: International Programme on Chemical Safety
NIEHS: National Institute of Environmental Health Science
OXFAM: Oxford Committee for Famine Relief
REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals
WHO: World Health Organization
UNEP: United Nations Environment Programme
US-EPA: United States Environmental Protection Agency

List of Figures

List of Tables

CHAPTER 1

GENERAL INTRODUCTION

Chapter 1. General Introduction

1.1. Human exposure to environmental contaminants

Anthropogenic activities are known to adversely affect the environment and human health. In fact, a vast number of chemicals that were originally introduced into the market to increase the quality of life and to improve human and animal health exhibit toxic potential. Several incidents that occurred during the 20th century launched the first associations between humans' adverse health effects and the exposure to chemicals. These incidents include, for example, (i) the phenomena known by the "Jamaican ginger paralysis" in the 1920s, which caused outbreaks of neurological illness in about 50 000 people. This incident was caused by tri-ortho cresyl phosphate used as adulterant in ginger drink; (ii) the landmark study in the United States (from 1911 to 1920) documented by Alice Hamilton on the occupational toxic disorders caused by the exposure to several chemicals, such as lead; (iii) the Minamata disease or "cat dancing disease" in Japan (during the 1950s), characterized by a neurological syndrome that was caused by methylmercury poisoning due to the consumption of contaminated fish in humans and animals' (particularly cats); (iv) the explosion at an herbicide plant in Seveso, Italy in 1976, which lead to the human exposure to high levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the victims suffered from chloracne among other adverse effects; (v) the methyl isocyanate explosion in Bhopal, India in 1984, causing the death of thousands people and caused health problems such as oedema in hundreds of thousands; (vi) the nuclear explosion in Chernobyl, Russia 1986, the eventual death toll, resulting from the exposure to radiation, varies between 4000 to 200 000, and with millions of people affected with various health problems, such as thyroid cancer, that still affects the population nowadays; (vii) the largest maritime oil spill in the history in the Gulf of Mexico in 2010, in which 780 000 m^3 of oil were spilled over 87 days, resulting in the accumulation of genotoxic substances in the air, soil and water; (viii) the Fukushima nuclear accident after the earthquake and tsunami in 2011, in Japan, where special health care programmes of the World Health Organization (WHO), based on the Chernobyl nuclear disaster, were implemented after the incident in order to avoid serious consequences, such as the increased risk of radiation-associated childhood thyroid cancers, however, the long term effects are still difficult to

predict and manage (Lioy, 1990; Gilbert, 2011; Danzer and Danzer, 2016; Singleton et al., 2016; Yamashita et al., 2016).

The aforementioned environmental disasters reflect the evolution of the chemical industry and agriculture (Szirmai, 2012) and, consequently the drastic increase in the use of chemicals. Thus, chemicals' emissions, distribution in the environment and accumulation also increased, affecting different environmental compartments, including air, water, land and aquatic sediments and biota. As a consequence, the concern over these contaminants emerged, particularly after Rachel Carson pioneer work. Rachel Carson was a marine biologist and environmentalist who contributed to the public environmental consciousness. She conducted research on the effects of pesticides in the food chain, especially dichlorodiphenyltrichloroethane (DDT). By 1962, she published the book "Silent Spring" where she described a series of harmful effects on the environment and wildlife caused by the use of DDT, including how DDT enters the food chain and accumulates in the fatty tissues of animals and humans, and may cause cancer and genetic damage (Carson, 1962).

Despite such evidences, the global production of chemicals continued to increase, raising from 1 million tonnes in 1930 to over 400 million tonnes by 2006 (REACH, 2006), and in 2011 the global output was valued at $ 5 trillion. Currently the exact number of chemicals in the market is unknown, in 2013 there were for example, 143 835 chemicals on the European Union (EU) market (Sousa et al., 2013), according to the preregister at the European Union Regulation on Chemicals, REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals).

Chemicals are used in a wide variety of processes and products designed to fulfil the demands of modern live, and therefore, there are a vast number of sources of environmental contamination and exposure to these man-made chemicals (Holt, 2000). Besides the contamination by the naturally occurring compounds, the major sources of anthropogenic chemicals are: industry sectors, burning/ incineration, cigarette smoke, commercial products and automobile exhaust (Tilley and Fry, 2015). The release of chemicals into the environment and the exposure to those chemicals may occur during different stages of their life cycle (Figure 1.1.1). During the extraction of the raw materials, during the manufacturing, processing or refining of the bulk and downstream chemical, during the use and reuse of the chemicals or the chemical containing-products or during

their recycling and disposal, resulting in two possible ways of exposure, occupational and environmental (UNEP, 2013).

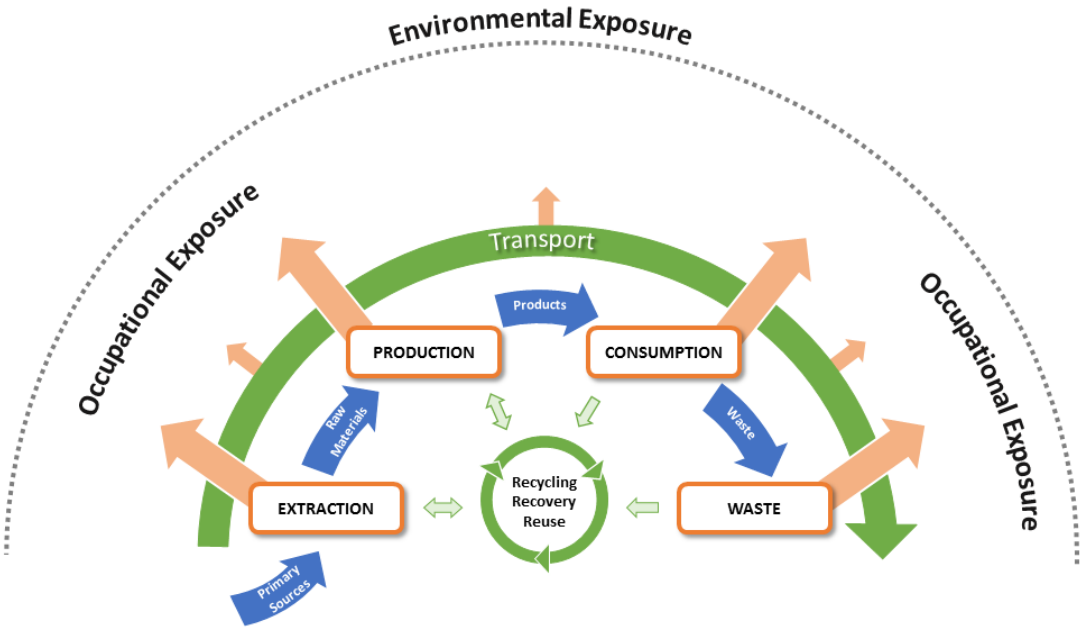

Figure 1.1.1. Life cycle of chemicals. Adapted from EEA (2010) and UNEP (2013).

Once released into the environment some chemicals can travel long distances so that exposures may occur near the sites of production and use or in remote locations. Some chemicals have short life-times in the environment, however they are frequently released in effluents, agricultural runoffs and from urban environments, and therefore their levels are high near the sources (WHO/UNEP, 2013). Other synthetic chemicals, the persistent ones, can be carried by air and water currents over long distances, being detected in unspoiled locations such as the Arctic and Antarctic. When released into warmer climates, they can evaporate into the atmosphere and be transported by the wind to cooler areas (including the Polar Regions) where they condense and deposit in water resources (Iwata et al., 1993; Wania and MacKay, 1996; Zhang et al., 2010), then they bioaccumulate in aquatic microorganisms and biomagnify along the food chain (Figure 1.1.2). Therefore, organisms at the top of the food chains tend to have higher concentrations of these contaminants stored in their fatty tissues (due to their strong affinity for lipids), such as seals, bears and also humans (Raven et al., 2012; UNEP, 2013; WHO/UNEP, 2013).

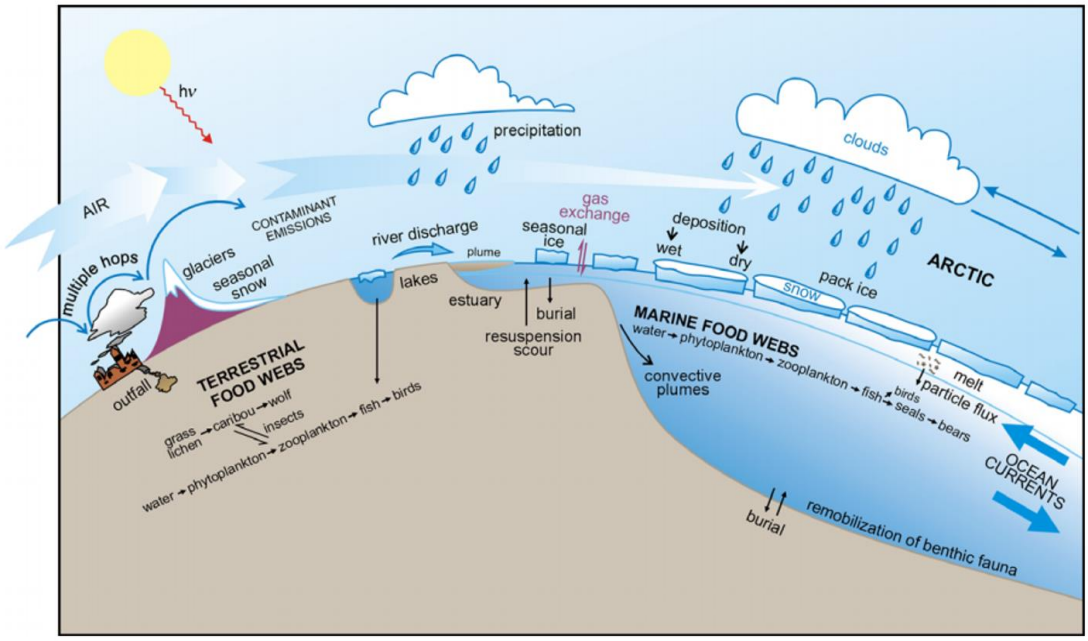

Figure 1.1.2. Transport processes for persistent organic pollutants that are responsible for the up-concentration in Northern environments. Source: Macdonald et al. (2005).

Besides the ingestion of contaminated food (from food production, or bioaccumulation along the food chain, or during the storage or processing) and given the countless sources of contaminants (e.g. cosmetics, pharmaceuticals, electric and electronic products, furniture and cleaning products), there are many routes of exposure to chemicals, such as the ingestion of water, soil and dust, the inhalation of air (gas and particles) and dermal contact (US-EPA, 2015a). Therefore, humans are extremely vulnerable being continuously exposed to a complex mixture of chemicals and this exposure has been associated to a large number of adverse health effects (e.g. cancers, cardiovascular disease, asthma and developmental disorders). However, little is still known about the total disease burden attributable to these environmental contaminants (UNEP, 2013). In 2004, the WHO estimated that, globally at least 8.3% of all preventable deaths and 5.7% of the preventable disability-adjusted life years (DALYs, a measure combining mortality and morbidity) were attributable to environmental exposure and management of selected chemicals (Prüss-Ustün et al., 2011).

1.2. Classes of environmental contaminants

The environmental contaminants can be categorized in different categories, including toxic metals, persistent organic pollutants (POPs) and contaminants of emerging concern (CECs).

Metals can be divided in two main groups, the essential elements required for life (e.g. iron and calcium) and those that are nonessential or toxic (e.g. lead and cadmium). Due to their physicochemical properties, these toxic metals have multiple applications with industrial, domestic, agricultural, medical and technological purposes (Nordberg et al., 2007a). Although they are naturally occurring compounds found throughout the earth's crust, their release and further environmental contamination results mostly from numerous anthropogenic activities such as mining and smelting operations, industrial and agricultural emissions and technological activities (Tchounwou et al., 2012). For a long time, metals are recognized as a major environmental problem (Mansour, 2011), and the concern on the deleterious effects associated to the exposure to these metals towards the wildlife and human health has been increasing. This global concern arises because these chemicals do not breakdown and accumulate in the environment especially in water and soils, furthermore they can be transported between environmental compartments (Mohammed et al., 2011), concentrate all the way to the top of the food chain and reach humans (Naja and Volesky, 2009). Unlike several organic compounds, metals are not metabolically degradable remaining in the body until they can be excreted. If they are not easily eliminated they accumulate in the living tissues and therefore they may cause serious health problems and even death (Nordberg et al., 2007a; Naja and Volesky, 2009). Furthermore, some metals are toxic even at extremely low concentrations (Cornelis and Nordberg, 2007). Table 1.2.1 discloses some of the health problems associated with metal exposure. Toxic metals such as arsenic, cadmium, chromium, copper, lead, mercury and nickel can interfere with the functioning of many important organs such as the brain, lungs, kidney, liver and blood composition. Long-term exposure can cause physical, muscular and neurological degenerative processes, and for some metals, a repeated long-term exposure may also cause cancer (Jaishankar et al., 2014).

Table 1.2.1. Types of metals and their adverse effects on human health.

Metal	Adverse effects on human health
Arsenic	Bronchitis, dermatitis, poisoning, cancer of lungs, liver, bladder and skin
Cadmium	Renal dysfunction, lung disease, lung cancer, bone defects (osteomalacia, osteoporosis), increased blood pressure, kidney damage, bronchitis, gastrointestinal disorder, bone marrow
Lead	Mental retardation in children, developmental delay, fatal infant encephalopathy, congenital paralysis, sensor neural deafness and acute or chronic damage to the nervous system, epilepticus, liver, kidney and gastrointestinal damage, cardiovascular effects, increased blood pressure
Mercury	Tremors, gingivitis, minor psychological changes, spontaneous abortion, damage to nervous system, protoplasm poisoning
Chromium	Damage to the nervous system, fatigue, irritability, cancer
Copper	Anemia, liver and kidney damage, stomach and intestinal irritation

Based on Singh et al. (2011) and Jaishankar et al. (2014) and Nordberg et al. (2007a).

Persistent Organic Pollutants (POPs) are synthetic carbon-based (organic) chemical substances that, once released in the environment, have the ability to accumulate, persist and bioconcentrate. These compounds persist for remarkably long periods of time, they are widely spread as a result of natural processes in soil, water and mainly air which leads to global pollution. They have high affinity for the adipose tissues of living organisms and accumulate through the food chain, therefore they are found at higher concentrations in higher levels in the food chain, including in humans (Stockholm Convention, 2015). POPs are toxic to both humans and wildlife. Their global distribution and contamination is due to the fact that they are semi-volatile, and therefore they can partially evaporate at high temperatures and be transported to higher latitudes, where the cold temperatures makes them condense and deposit from the atmosphere onto soil and water (Wania and MacKay, 1996) (Figure 1.1.2). This group of compounds comprises mainly synthesized substances of agricultural and industrial uses and by-products generated from human and natural activity (Stockholm Convention, 2015). Due to their high toxicity and widespread environmental distribution the use of POPs is regulated by the United Nations, under the Stockholm Convention. This Convention signed in 2001, and effective May 2004, initially focused on the reduction or elimination of the production, use and release of 12 highly toxic chemicals, so-called "Dirty dozen" (Table 1.2.2).

More chemicals were subsequently added to the list of POPs during the Stockholm Convention meetings in recent years. (Table 1.2.3).

These organic chemicals are known to cause several specific adverse health effects, including allergies and hypersensitivity, damage to the central and peripheral nervous system, reproductive disorders, birth defects and damage to the respiratory and immune systems. Some POPs are considered carcinogens and some even act like endocrine disruptors (Li et al., 2006; Mansour, 2011; Stockholm Convention, 2015).

Table 1.2.2 Persistent organic pollutants: The "Dirty dozen".

Chemical	Use	Stockholm Convention Annex[a]
Aldrin	Insecticide	**A**
Chlordane	Insecticide	**A**
Dichlorodiphenyltrichloroethane (DDT)	Insecticide	**B**
Dieldrin	Insecticide	**A**
Endrin	Insecticide and Rodenticide	**A**
Heptachlor	Insecticide	**A**
Hexachlorbenzene (HCB)	Insecticide and fungicide; By-product of incineration and industrial processes	**A, C**
Mirex	Insecticide; flame retardant	**A**
Toxaphene	Insecticide	**A**
Polychlorinated biphenyls (PCBs)	Industrial chemical	**A, C**
Polychlorinated dibenzo-p-dioxins (PCDD)	By-product of incineration and industrial processes	**C**
Polychlorinated dibenzofurans (PCDF)	By-product of incineration and industrial processes	**C**

Source: Stockholm Convention (2015)
[a] **Annex A** substances: slated for "elimination" in the Stockholm Convention. **Annex B** substances: slated for "restriction" for which there is a specified "acceptable purpose"; **Annex C** substances: continuing minimization and, where feasible, ultimate elimination of the total releases derived from anthropogenic sources.

Contaminants of Emerging Concern (CECs) are synthetic or naturally occurring chemicals or microorganisms for which concerns about their potential to pose risks to the environment and human health were raised more recently (Raghav et al., 2013; Sauvé and Desrosiers, 2014). These contaminants include not only those new chemicals recently produced and released in the environment, but also some chemicals already in use for a long time but for some reason not studied until recently. Therefore, there is limited data on their occurrence, environmental fate, and toxicity (Meador et al., 2016). The list of CECs includes substances for which no maximum levels have been set in the legislation, and identified compounds for which maximum levels have already been set but are still under revision due to new hazard information (Vandermeersch et al., 2015).

Table 1.2.3. New persistent organic pollutants.

Chemical	Use	Stockholm Convention Annex[a]
Alpha hexachlorocyclohexane (α-HCH)	Pesticide; By-product of incineration and industrial processes	**A**
Beta hexachlorocyclohexane (β-HCH)	Pesticide; By-product of incineration and industrial processes	**A**
Chlordecone	Pesticide	**A**
Hexabromobiphenyl	Industrial chemical	**A**
Hexabromocyclododecane (HBCDD)	Industrial chemical	**A**
Hexabromodiphenyl ether and Heptabromodiphenyl ether (hexaBDE and heptaBDE)	Industrial chemical	**A**
Hexachlorobutadiene	Industrial chemical	**A**
Lindane	Pesticide	**A**
Pentachlorobenzene	Pesticide; Industrial chemical; By-product of incineration and industrial processes	**A, C**
Pentachlorophenol and its salts and esters	Pesticide	**A**
Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride	Industrial chemical	**B**
Polychlorinated naphthalenes	Industrial chemical; By-product of incineration and industrial processes	**A, C**
Technical endosulfan and its related isomers	Pesticide	**A**
Tetrabromodiphenyl ether and pentabromodiphenyl ether (tetraBDE and pentaBDE)	Industrial chemical	**A**

Source: Stockholm Convention (2015)
[a] **Annex A** substances: slated for "elimination" in the Stockholm Convention. **Annex B** substances: slated for "restriction" for which there is a specified "acceptable purpose"; **Annex C** substances: continuing minimization and, where feasible, ultimate elimination of the total releases derived from anthropogenic sources.

These compounds will remain as "emerging" as long as the scientific information is scarce and the number of documented issues on their potential risk is limited. However, a contaminant which have already been regulated and well described can recover the "emerging" status if new scientific information becomes available, forcing the re-evaluation on their norms and guidelines by the regulatory agencies (Sauvé and Desrosiers, 2014). Due to their large diversity and ubiquity, they are frequently separated into categories according to their nature, purpose, use and other characteristics, such as surfactants, synthetic hormones, pharmaceuticals and personal care products, plasticizers, flame retardants and pesticides (Table 1.2.4).

Table 1.2.4. Categories of contaminants of emerging concern.

CECs category	Chemical example	Definition
Antibiotics	Tetracycline, Erythromycin	Medications that fight bacterial infections, inhibiting or stopping bacterial growth
Detergent metabolites	Nonylphenol	Chemical compounds formed when detergents are broken down by wastewater treatment or environmental degradation
Estrogenic compounds	Estrone, Estradiol, Nonylphenol, Bisphenol A	Natural or synthetic chemicals that can elicit an estrogenic response
Flame retardants	Polybrominated Diphenyl Ethers (PBDEs)	Any of several materials or coatings that inhibit or resist the spread of fire
Fragrances	Galaxolide	Chemical substances that impart a sweet or pleasant odour
PAHs (Polycyclic aromatic hydrocarbons)	Benzo(a)pyrene, Fluoranthene, Naphthalene	Chemical substances usually found in the environment as a result of incomplete burning of carbon-containing materials like fossil fuels, wood or garbage
Personal Care Products	Para-hydroxybenzoate	Chemical substances used in a diverse group of personal items including toiletries and cosmetics
Pesticides or Insecticides	Permethrin, Fenitrothion, Bacillus thuringiensis israelensis	Chemical substances or microbiological agents that kill, incapacitate or otherwise prevent pests from causing damage
Pharmaceuticals	Fluoxetine, Carbamazepine, Diphenhydramine	Chemical substances used in the prevention or treatment of physiological conditions
Plasticizers	Dioctyl Phthalate (DOP)	Chemical additives that increase the plasticity or fluidity of a material
Steroids	Cholesterol, Coprostanol, Estrone, Progesterone	Fat-soluble organic compounds with a characteristic molecular structure, which includes many natural and synthetic hormones
Surfactant	Sodium Lauryl Sulfate, Perfluorooctane sulfonate (PFOS) and Perfluorooctanoic acid (PFOA)	Chemical substances with an exceptional stability and capacity for lowering surface tension affecting the surface of a liquid

Based on Raghav et al. (2013) and Vandermeersch et al. (2015).

Beyond these categories (toxic metals, POPs and CECs), the environmental contaminants might be distributed in other classes which can vary according to many different characteristics, such as their physicochemical nature, origin, use, fate and behaviour in the environment, their ecological impact and effects in the human health. Therefore, many contaminants can match into various classes as for example several flame retardants and highly fluorinated chemicals.

Considering the adverse health effects, one of the most important classes is the one associated with endocrine disrupting chemicals (EDCs), which, according to the Endocrine Society (2015), *are defined as chemicals, or mixture of chemicals, that interfere with any*

aspect of hormone action, consequently they may affect health and reproduction in animals and humans. These chemicals are largely manufactured for specific industrial purposes (flame retardants, plasticizers, pesticides and food-packaging, etc.) (Table 1.2.5), and are highly diverse: they can be metals or organic compounds, highly persistent or not, large or small molecules and soluble in fat or water (Casals-Casas and Desvergne, 2011; WHO/UNEP, 2013; Endocrine Society, 2015). Due to their great diversity, EDCs are ubiquitous being present in the environment and for example in consumer products, food and food storage containers and personal care products (Kabir et al., 2015).

Table 1.2.5. Examples of endocrine disrupting chemicals and their uses.

Common EDCs	Uses
DDT, HCB, atrazine	Pesticides
Lead, cadmium	Children's products, ceramics, glasses
BPA, phthalates, phenol	Food contact materials
Brominated flame retardants, PCBs	Electronics and building materials
Phthalates	Personal care products, medical tubing
Perfluorochemicals	Textiles, clothing, kitchen utensils
Parabens, phthalates, glycol ethers, fragrances, cyclosiloxanes	Cosmetics, personal care products, cleaners
Tributylin	Antifouling biocides used to paint the bottom of the ships
Nonylphenol (alkylphenols)	Surfactants-certain kinds of detergents used for removing oil and their metabolites

Based on Kabir et al. (2015).

EDCs can interfere with several biochemical ways, they can mimic or block the biological activity of natural hormones, as well as interfere with their production, release, metabolism and elimination (Casals-Casas and Desvergne, 2011). Hence, they are associated with many adverse health effects, including the disruption of the thyroid and the corticoid functions (e.g. diabetes, obesity and pituitary deregulation), disruption of the nervous system (e.g. effects on the behaviour, learning, memory, attention, sensory function and neurological development) and effects on male and female reproduction (e.g. increased growth of endometrium, ovarian failure, irregularities in menstruation cycle, decline in the sperm quality and decreasing fertility) (Kabir et al., 2015). In more severe cases, they have also been associated with higher risk to develop breast, endometrial, ovarian, prostate, testicular and thyroid cancers (WHO/UNEP, 2013).

1.3. Contaminants under the scope of this thesis

1.3.1. Flame retardants

Flame retardants (FRs) are chemicals added to a large range of products to increase their flame ignition resistance. The use of organic brominated, chlorinated and phosphate FRs expanded since the early 1970s, especially in the United States (US), due to the increasing fire safety regulations in order to compensate the increased use of flammable materials, such as plastics in electrical equipment or synthetic fibres (Babrauskas et al., 2014). These compounds contributed greatly to reduce the risk of fires and the incidence of fire injuries and mortality, however, beyond these benefits FRs are associated with environmental health issues (Blum, 2007).

One of the first obvious examples of the hazards of FRs was the poisoning incident with polybrominated biphenyls (PBBs) in Michigan, US in 1973. The commercial mixture of PBB was inadvertently packed into bags similar to those of magnesium oxide feed additive, being added to animal feed. This contamination resulted in the loss of livestock and long-term impacts on the health of local farm families who have eaten large amounts of dairy products and eggs from the affected animals (Blum and Ames, 1977; Birnbaum and Staskal, 2004). As a consequence, this FR was banned in the US in 1976 (US-EPA, 2014). Still in the 1970s, other two FRs which were widely added to polyester children's pyjamas, tris(2,3- dibromopropyl) phosphate (tris-BP) and tris(1,3-dichloro-2- propyl) phosphate (TDCIPP), raised concern after being identified as mutagenic and potentially carcinogenic (Blum and Ames, 1977; Gold et al., 1978). These concern emerged mainly because they could be absorbed from the fabrics by children's body after a single night of sleep (Blum et al., 1978). Consequently, their use in children's sleepwear was banned by the Consumer Products Safety Commission. Nevertheless, this limited regulation allowed these FRs to be applied in other products such as polyurethane furniture foams. As a consequence three decades after, TDCIPP was the second most used FR in furniture foams as confirmed by Blum (2007).

In the last decade, brominated flame retardants (BFRs) have received much regulatory attention, especially polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs). By 2000, BFRs accounted for 38% of the global

bromine demand, whilst in 1975 they accounted only for 8% (Birnbaum and Staskal, 2004). This group of FRs interferes with the combustion process through gas phase chemical reactions, thus delaying the ignition and inhibiting the spread of fire (US-EPA, 2015b). These characteristics promoted their use in a large number of consumer products including textiles, polyurethane foams, upholstery for furniture and car seats, electronic and electric devices, plastics (for televisions, computers and other electronic equipments) and building materials. PBDEs were mainly used as additive FRs (US-EPA, 2015b) in electronic devices like computers and TV sets, upholstery and carpets, while HBCDD (also used as additive compound) was primarily used in polystyrene foams and upholstery textiles (Fromme et al., 2015). Since they are mainly used as additive FRs, they are physically mixed with the material polymers without undergoing any chemical reaction, and therefore they are likely to be released from the products over time and use (Stapleton et al., 2008).

As the result of the widespread production, use and release of BFRs, strong evidences of increasing contamination of the environment and even humans emerged through time. PBDEs and HBCDDs have been recognised as lipophilic compounds as well as persistent, bioaccumulative and toxic. Furthermore, they undergo long-range atmospheric transport (tetra to heptaBDEs and HBCDD are classified as POPs, Table 1.2.3) (Birnbaum and Staskal, 2004; Coelho et al., 2014). The decaBDE congener (highly brominated congener) is not categorized as POP, however it can degrade to lower brominated PBDEs when exposed to sunlight (EFSA, 2011; Kalachova et al., 2012). Due to these characteristics, the commercial mixtures pentaBDE (composed mainly by BDE 47, 99 and 100) and octaBDE (predominantly BDE 153, 154 and 183) were phased out in the European Union and the US in 2004; and decaBDE (mainly composed by the BDE 209 congener) was banned from electric and electronic devices in Europe in 2008, and phased out in the US in 2013 (Coelho et al., 2014).

After the implementation of the legislation on PBDEs and HBCDDs, there was a need to replace them with other FRs. Therefore the demand of emerging BFRs (EBFRs) and phosphorus flame retardants (PFRs) increased in the last several years. In fact, there has been an increase in the use of 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) and decabromodiphenyl ethane (DBDPE), which replaced octaBDE and decaBDE, respectively (Newton et al., 2015). Although these EBFRs' properties and effects in the environment

are mostly unknown, their physicochemical properties and structure are similar to the PBDEs they replace, and therefore they appear to have similar toxicological behaviour (Babrauskas et al., 2014; Newton et al., 2015). Several studies have been developed in order to investigate their occurrence and environmental behaviour (Covaci et al., 2011).

PFRs are FRs with no or low bioaccumulative potential, and their mechanism of action can also be through gas phase reactions, but it occurs mostly in the solid phase of the burning materials (van der Veen and de Boer, 2012), by forming a carbonaceous layer on the surface of the polymer, which interferes with the transfer of heat from the gas phase to the solid phase preventing the thermal decomposition (US-EPA, 2015b). Some are additive FRs such as BFRs. Other, however, are reactive, being covalently bound to the materials, which renders their release into the environment less likely (van der Veen and de Boer, 2012). As all FRs, their application is varied, PFRs are extensively used in building materials, interior decoration, furniture, upholstery, electrical and electronic materials and transport vehicles (Mizouchi et al., 2015). Organic PFRs are divided in two main classes (Chen et al., 2012), the halogen containing PFRs such as tris(2-chloroethyl) phosphate (TCEP) and tris (1,3-dichloro- 2- propyl) phosphate (TDCIPP); and the non-halogenated PFRs, including tripropyl phosphate (TPP), tri-n-butyl phosphate (TNBP), 2-ethylhexyl diphenyl phosphate (EHDPP), tricresyl phosphate (TMPP), tripentyl phosphate (TPEP), triphenyl phosphate (TPHP) and tris(2-ethylhexyl) phosphate (TEHP) (Table 1.3.1).

TPP and TDCIPP are used as replacement of pentaBDE (Babrauskas et al., 2014), the last being the one banned from pyjamas in 1978, and TPHP is a substitute for decaBDE (van der Veen and de Boer, 2012).

Recently, the same concern on the development of alternatives for BFRs, also emerged for halogen containing PFRs, and the substitution with non-halogenated PFRs is being considered. Mainly because their boiling points are higher and therefore they are less volatile and thus less likely to be released from materials (van der Veen and de Boer, 2012).

Many studies have been conducted over the last decade in order to evaluate the release, fate and toxicity of all mentioned FRs, even the ones already phased out. Much of the products containing the banned FRs are still in use, and therefore FRs will remain in circulation, being able to be released for a long period of time to come.

Table 1.3.1. Examples of phosphorous flame retardants and their uses.

PFRs	Application
Halogenated PFRs	
Tris(2-chloroethyl) phosphate (TCEP)	PVC, cellulose, coatings, polyester resins, textile, polyurethane foam
Tris (1,3-dichloro- 2- propyl) phosphate (TDCIPP)	Plastic, textile, polyurethane foam
Non-halogenated PFRs	
Tripropyl phosphate (TPP)	Polyurethane foam
Tri-n-butyl phosphate (TNBP)	Varnish, concrete, glue and airplane hydraulic fluids
2-ethylhexyl diphenyl phosphate (EHDPP)	Plastics, polyurethane foam, paints, glues, lacquers and varnishes
Tricresyl phosphate (TMPP)	Hydraulic fluids, PVC, cellulose, cutting oils, plastic, polystyrene, thermoplastics, transmission fluids, solvent
Tripentyl phosphate (TPEP)	Plastics, polyurethane foam, paints, glues, lacquers and varnishes
Triphenyl phosphate (TPHP)	Hydraulic fluids, PVC, electronic equipment such as video display units cables, casting resins, glues, engineering thermoplastics, phenylene-oxide-based resins, phenolics resins
Tris(2-ethylhexyl) phosphate (TEHP)	PVC, cellulose, paints and coatings, rubber, solvent, polyurethane foam

Based on Kim et al. (2011), van der Veen and de Boer (2012) and Brandsma et al. (2014).

1.3.2. Polychlorinated biphenyls and Organochlorine pesticides

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) are known as hazardous chemicals for human health for decades.

PCBs are in the list of the twelve chemicals firstly classified as POPs (Table 1.2.2), and they result largely from anthropogenic activities. They had great industrial and commercial applicability due to their non-flammable properties, chemical stability, high boiling point, low heat conductivity and high dielectric constants (EFSA, 2005). Hence, their mainly use was as dielectric oil in transformers, capacitors and paint (Cairns and Siegmund, 1981). PCBs were also extensively used in other products including carpets and upholstery, sealants, coolants, lubricants, pesticides and electronic equipment (Hinwood et al., 2014).

The contamination by PCBs was first discussed in 1969, when a Swedish scientist detected PCBs in fish and birds (Jensen et al., 1969), confirming that their environmental spread led to wildlife contamination. With this, the concern on the indirect contamination of certain

foods emerged. Then, due to their bioaccumulative potential, PCBs were found in human adipose tissue and human milk (Cairns and Siegmund, 1981).

Although their commercialization was discontinued in almost all industrial countries since the late 1980s, PCBs are still being released from many products in use and will persist in the environment for long periods of time, as well as in human body, due to their long half-lifes that varies from days to years depending on the structure and degree of chlorination of each congener (EFSA, 2005).

In order to follow the exponential increase of the world population and the consequent need for food production, pesticides have been widely used in order to protect the agricultural crops from pests since the 1940s (Grung et al., 2015). They can be classified according to their target species, for example herbicides, insecticides and fungicides. Hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), chlordanes (CHLs) and dichlorodiphenyltrichloroethane (DDT) are examples of OCs that, such as PCBs, are in the "Dirty dozen" list of POPs (Table 1.2.2). The concerns over risk associated with the use of pesticides emerged with the findings about DDT contamination described in the "Silent Spring" book published in the 1960s (subchapter 1.1.), and the first report of OCs contamination in arctic marine mammals by the end of the same decade (Holden, 1970; Norstrom and Muir, 1994). As a consequence, the regulation on the chemical pesticides use was strengthened. Although the consumption of OCs has been phased out in most countries since the 1970s and 1980s, these compounds can still be detected in the environment, animals and humans (Saravi and Dehpour, 2016).

1.3.3. Toxic metals

As previously mentioned, metals are also chemicals of concern. Even the metals considered as essential for humans such as cobalt, copper, iron, magnesium, manganese, molybdenum, selenium, and zinc, they can also be toxic if the exposure is excessive (Becking et al., 2007). For some of them, such as chromium and copper, the beneficial concentrations are very close to those that can generate toxic effects in humans (Tchounwou et al., 2012). Nickel, which is not known as essential to human health but may have some beneficial effects at low levels (Goyer, 2004), is also in the list of toxic metals. It was, along with chromium, the first metal classified as carcinogen to humans by

the International Agency for Research on Cancer (IARC, 1990). Other metals such as arsenic, cadmium, lead and mercury are nonessential metals with recognized toxicity (Jaishankar et al., 2014). Wherein, lead (Pb) and cadmium (Cd) are two of the most studied ones concerning their use, release, spread, human exposure and associated human health risks (Figure 1.3.1). In the list of priority substances set by the Agency for Toxic Substances and Disease Registry (ATSDR, 2015), Pb occupies the second position and Cd the seventh position of the ranking.

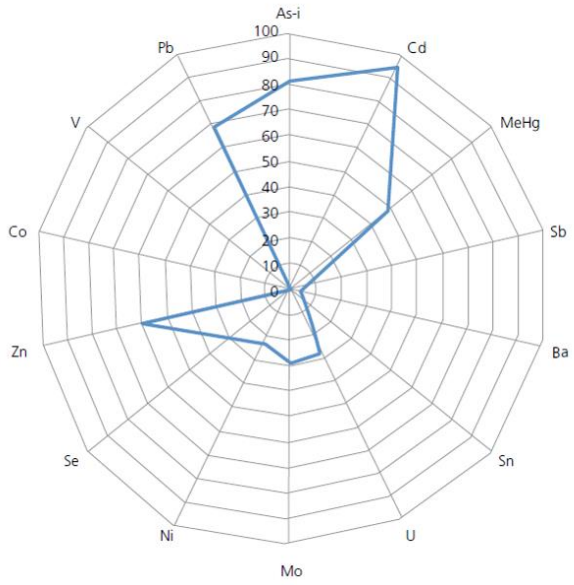

Figure 1.3.1. Risk characterization (% health based guidance values) for relevant metals. Source: Yusà and Pardo (2015).

Pb was predominantly used in vehicles, electric systems and industrial batteries, paints, water piping and as antiknocking agent in gasoline and therefore the main sources of lead include: leaded gasoline, industrial emissions and lead based paints (Skerfving and Bergdahl, 2007). Pb is a highly poisonous metal which affects almost every organ and systems in the human body (Pavón et al., 2015). The greatest percentage of Pb is taken into the kidney, followed by the liver and other soft tissues, however it accumulates mostly in bones (stored for decades), thus Pb in the skeleton represents the major body fraction (EFSA, 2010). The most critical target organ for Pb is the central nervous system, especially in developing brains. Pb is known by the potential to cause neurological and cognitive disorders, increased blood pressure and cardiovascular and renal diseases (EFSA, 2010; Pavón et al., 2015). Inorganic Pb has been classified has probably carcinogenic to humans (IARC, 2006).

Due to the high body burdens of Pb in the modern society (Skerfving and Bergdahl, 2007) and the consciousness of its adverse effects, its use was significantly reduced since the 1970s in developed countries. Such decreases were associated with the phased out of lead in gasoline and paints (Yusà and Pardo, 2015). Despite the well-known adverse risks, the use of Pb has continued in developing countries, where the disposal centres, for new and used leaded products exported from industrialised countries, are often located. This leads to serious local and regional environmental contamination as well as health risks to the people that use, manage and recycle or dispose these products (US-EPA, 2010). Overall, due to numerous hazards and environmental ubiquity, Pb is still a matter of great concern even in the countries with strict legislation.

Cd is used globally as a component in various products. It has been used for electroplating, as an anticorrosive, it is used in paint pigments, as stabilizer for plastics and it was widely used in the manufacture of nickel-cadmium batteries (Nordberg et al., 2007b; WHO, 2010). The environmental and health concerns over cadmium exposure led to a decline in the commercial use of this metal in developed countries (Tchounwou et al., 2012). In the European Union (EU), cadmium use decreased considerably during the 1990s as a consequence of the reduction in the manufacture of nickel-cadmium batteries and also due the implementation of more restrictive legislation, e.g. Directive 91/338/EEC. This directive was an amendment of Directive 76/769/EEC that restricted the marketing and use of certain dangerous substances and preparations, and added Cd to this list. In the human body, Cd accumulates primarily in the kidney, considered as the critical target organ, where this metal is efficiently retained (half-life 10 to 30 years) with levels proportional to those in urine (Pavón et al., 2015). It is also efficiently retained in the liver (EFSA, 2012). Long-term exposure to Cd can cause several adverse effects being the major ones renal tubular dysfunction, osteoporosis and osteomalacia, chronic obstructive lung disease and lung cancer (Nordberg et al., 2007b). Furthermore, there is sufficient evidence for their carcinogenicity in humans, and therefore Cd and cadmium compounds are considered carcinogenic to humans (Group 1) (IARC, 2012).

Once released into the environment, Pb and Cd stay in circulation because they are not degradable in nature, and once in the human body, they will be stored for decades leading to a large range of health complications. They are, therefore, relevant harmful metals whose extensive research remains important.

1.4. Major exposure sources and pathways

The increasing number of studies describing measurable body burdens of environmental contaminants associated to the growing epidemiological evidence of human health effects led to primary question of how humans are exposed to these compounds.

According to the Environmental Protection Agency (US-EPA, 2015a), the risk assessment of chemicals follows 4 major steps:

- Hazard identification;
- Dose-response assessment;
- Exposure assessment;
- Risk characterization.

The exposure assessment is "*the process of estimating or measuring the magnitude, frequency, and duration of exposure to an agent, along with the number and characteristics of the population exposed. Ideally, it describes the sources, routes, pathways, and uncertainty in the assessment*" (IPCS, 2004).

Considering this important step, the human exposure to the mentioned compounds (BFRs, EBFRs, PFRs, PCBs, OCs, Pb and Cd) may have a countless number of sources. Beyond their release from the industrial, commercial and domestic products where they are used (see previous sections for details), they are also released during the chemicals' and products' manufacture, their recycling and disposal (Figure 1.1.1). Therefore, these contaminants are reported to occur in the environmental compartments such as air, water, soil, sediments and biota and, especially, in the effluents of industrial plants and or waste disposal plants (wastewater, incinerators) (EFSA, 2014).

The exposure to chemicals may occur by three routes: inhalation, ingestion and dermal penetration. Inhalation includes the inhalation of dust, vapours, aerosols and fibers present in the contaminated air. Ingestion includes the ingestion of contaminated food, water and other drinks, and unintentional ingestion of contaminated soil and dust or residual chemicals on objects or surfaces which are ingested via object-to-mouth or hand-to-mouth activity (affecting mainly young children). Dermal penetration occurs through the skin contact with contaminated environmental media (water, sediment, soil and dust) (US-EPA, 2015a). Due to the numerous sources and various routes, the exposure might occur in

different moments of the chemicals life cycle and in different locations, thus the exposure can be classified as:

- occupational exposure
- non-occupational exposure
- outdoor exposure
- indoor exposure

Many studies assessed the occupational exposure by evaluating the levels and the associated adverse effects in the human body. Considering the BFRs, several studies revealed elevated serum concentrations of PBDEs in workers from an electronic recycling plant (Sjodin et al., 1999), electronic technicians (Jakobsson et al., 2002), carpet installers (Stapleton et al., 2008) and fire fighters (Shaw et al., 2013). For PCBs, associations were found, for example, between employment in three electrical capacitor manufacturing plants and increased intestinal cancer mortality among female long-term workers and excess melanoma mortality for male long-term workers (Ruder et al., 2014). As for toxic metals, Cd was classified as human carcinogen (Group 1) on the basis of many occupational studies (IARC, 2012).

Regarding the non-occupational exposure, the ingestion of food and ingestion of dust have been extensively discussed as major pathways of exposure to BFRs, EBFRs, PFRs, PCBs, OCs, Pb and Cd. Likewise, the human exposure to indoor contaminants has gained more significance over the years, since the general population tends to spend a large part of their lifetime indoors. These major pathways are describe in the next sections.

1.4.1. Diet – Ingestion of food

The ingestion of food is one of the leading causes of exposure to contaminants. Several factors are responsible for the contamination of food. During the production of foodstuffs, for example, contamination by hormones and steroids used in livestock production, by pesticides and fertilizers used directly in the crops or released from contaminated soils (e.g. OCs), by antibiotics used in animal production may occur. Furthermore, bioaccumulation along the food chain of fat soluble agricultural and industrial chemicals (e.g. OCs, BFRs, PCBs), may also occur. Other potential food contamination opportunities occur during the preservation and packing processes with contamination by chemicals added to preserve the

food (e.g. additives) and others released from food packaging, such as plastic storage containers, cling films and cans (e.g. phthalates and BPA). Additionally, chemicals released from the cooking utensils during the food preparation (e.g. PFCs); and chemicals created during the food processing (Connolly, 2009) (Figure 1.4.1) are also important. Therefore, food can concentrate many chemicals that will be ingested by humans and that may interfere with humans' health.

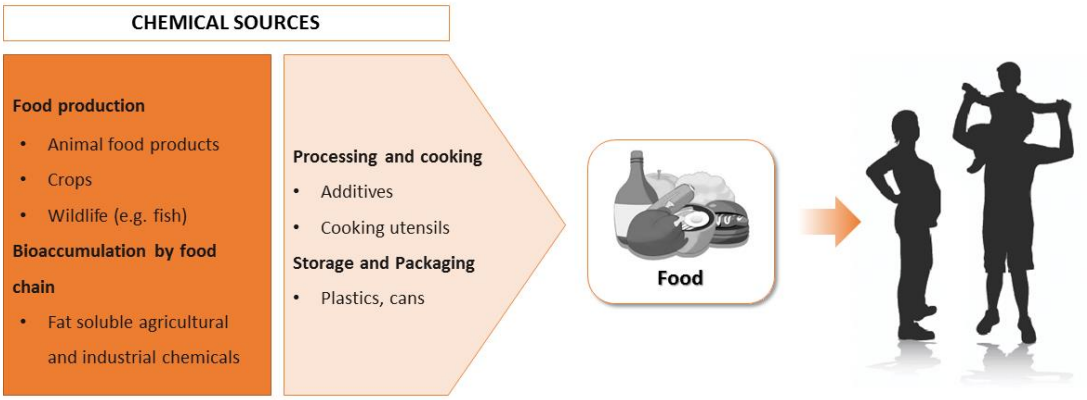

Figure 1.4.1. Contamination of the food from various sources. Based on Connolly (2009).

During the last decade, the number of studies evaluating the dietary intake of FRs, mainly BFRs (Schecter et al., 2006; Voorspoels et al., 2007; Driffield et al., 2008; Roosens et al., 2009; Fernandes et al., 2016; Martellini et al., 2016) has been growing. These studies confirm that despite some differences in the concentrations between countries, FRs are present in the diet on a worldwide basis. As a result of their bioaccumulative and fat solubility properties, the main contributors to BFRs in the diet are meat, fish and dairy products (Roosens et al., 2009; Domingo, 2012). Positive associations were even found between the consumption of dairy products and meat and the levels of PBDEs detected in human breast milk samples (Wu et al., 2007). These animal origin foodstuffs, are also the main sources of PCBs (Loutfy et al., 2006; Malisch and Kotz, 2014) and OCs (Kannan et al., 1997) in the human diet. Their levels in farm products, such as food grain and vegetables, decreased over time after they were phased out, however they are still present in the environment, mainly in animal products. Dietary intake of PCBs and OCs is estimated to account for over 90% of human exposure (Halldorsson, 2012).

Regarding the toxic metals, the consumption of food is also identified as the major exposure route to humans, it also accounts for over 90% comparing to the other pathways (inhalation and dermal contact) (Bortey-Sam et al., 2015). Many studies revealed the ubiquity of Cd and Pb in food (Nasreddine et al., 2010; Becker et al., 2011; Martorell et al., 2011; Islam et al., 2015; Koch et al., 2016). For metals, besides the animal origin foodstuffs, vegetables, cereal products and fruits are also important contributors (Norton et al., 2015; Koch et al., 2016) for the human exposure, due to the substantial contamination of soils and atmosphere (Ali and Al-Qahtani, 2012).

1.4.2. Dust – Ingestion of house dust

The ingestion of outdoor and indoor dust is an important pathway of exposure to environmental contaminants with diverse origins. Mostly because besides the inhaled dust humans may involuntary ingest considerable amounts of dust, central tendency of 30 mg day^{-1} estimated for adults and 60 mg day^{-1} for children (US-EPA, 2011). The higher amounts consumed by children are due to the fact that they spend much of their time playing on the floor and have the tendency to place objects and their hands on the mouth. For example, the hand-to-mouth behaviour is a major pathway for Pb exposure in young children (Moya and Phillips, 2014).

Over the years, the indoor dust has been considered as an important tool to assess the exposure of humans to contaminants, especially the persistent ones. This is the result of a global concern about the indoor environment quality which emerged because, in modern society people spent approximately 90% of their time indoors, including houses, workplaces, schools and public spaces (Le Cann et al., 2011), wherein, about 2/3 of that time is spent at home. House dust is a complex mixture of organic and inorganic matter and it works as a receptor and concentrator for various toxic chemicals from many sources (Figure 1.4.2), such as the degradation of building materials; the use of cleaning products, cosmetics, biocides, textiles, house furnishings, electric and electronic devices, and other household products; indoor activities (e.g. cooking, smoking, burning incense and candles); and intrusion of materials brought from outdoors (Mercier et al., 2011). The levels of chemicals in dust may be long-term predictors of indoor exposures (Whitehead et al., 2011), due to its repository function.

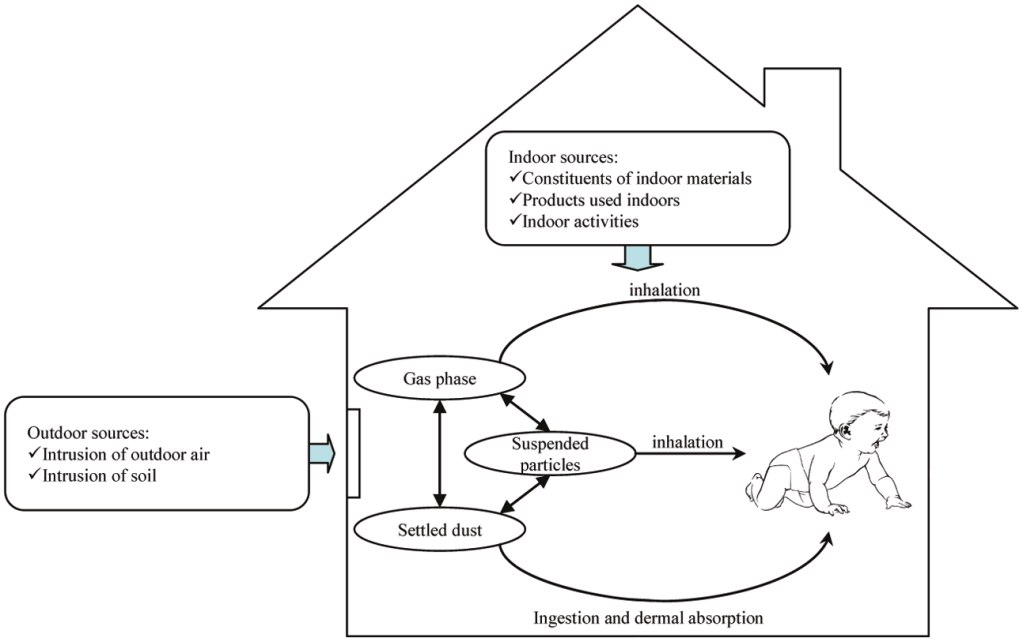

Figure 1.4.2. Sources of indoor contamination and pathways of indoor exposure. Adapted from Mercier et al. (2011).

Numerous chemicals have been measured in house dust worldwide. Many studies evaluated the levels of FRs, pesticides, phthalates, PAHs, PCBs and PFCs, however other organic contaminants have been detected in house dust, such as alkaloids and alkylphenols, dioxins and furans, musk fragrances, organophosphate esters, organotin compounds, parabens, and polychlorobenzenes (Mercier et al., 2011). The levels of inorganic compounds in house dust are also of great concern, such as toxic metals (Hogervorst et al., 2007; Quintana et al., 2008; Ibanez et al., 2010).

Besides the quantification of FRs in house dust, many studies found positive associations between these levels to those detected in human tissues (e.g. blood serum and plasma, breast milk and placenta), confirming the human exposure through indoor dust and their occurrence in the human body. For few congeners of PBDEs, the levels found in house dust were associated to the levels detected in blood plasma (Karlsson et al., 2007), blood serum (Stapleton et al., 2012), breast milk (Wu et al., 2007) and umbilical cord plasma (Frederiksen et al., 2010). HBCDD levels in indoor dust were also positively associated to the concentrations in human blood serum (Roosens et al., 2009), and related to altered hormonal levels in blood serum from men (Johnson et al., 2013). Significant correlations

were found between the levels of DBDPE and BTBPE in dust and those detected in human hair (Zheng et al., 2011).

Probably due to their increased use and due to the low or not existent bioaccumulative potential (van der Veen and de Boer, 2012), the number of studies for the PFRs is limited. However, positive associations were found between some PFRs levels in dust and human hair, as well as, between their levels in the hair and those of their metabolites in the urine (e.g. TDCIPP) (Kucharska et al., 2015). Some urinary metabolites concentrations were also correlated to their PFRs precursors in dust (Cequier et al., 2015).

For Cd and Pb, positive associations have also been found between the levels in dust and in human blood (Noonan et al., 2003; Hogervorst et al., 2007) and dust Pb levels are considered the best predictor of blood Pb levels in young children (Gulson et al., 2013).

Given the described environmental contamination and the uninterrupted human exposure to a vast number of chemicals and mixtures of chemicals and their deleterious effects, it is evident that both the environment and human health are affected. Therefore, it is crucial to study and understand which are the main sources of the contaminants and pathways of exposure in order to established preventive measures and therefore to reduce exposure. The present thesis aims to provide such scientific data by, as mentioned initially, characterizing the human exposure in Portugal to several contaminants, through two major pathways, the ingestion of food and the ingestion of house dust.

1.5. Aims and Rationale of the thesis

In recent years much attention has been given to the importance of the diet and house dust as sources of human exposure to several organic and inorganic toxic chemicals. Based on this concern, the main purpose of this thesis is to characterize the human exposure to several environmental contaminants and the associated risks through the ingestion of food and the ingestion of house dust in Portugal. The contaminants addressed in the present thesis include:

- Flame retardants (FRs):
    - Brominated flame retardants (BFRs):
        - polybrominated diphenyl ethers (PBDEs),
        - hexabromocyclododecanes (HBCDDs);

  - Emerging brominated flame retardants (EBFRs):
    - decabromodiphenyl ethane (DBDPE),
    - 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE),
    - 1,2-dibromo-4-(1,2-dibromoethyl)- cyclohexane (TBECH);
  - Phosphorus flame retardants (PFRs):
    - tris(2-chloroethyl) phosphate (TCEP),
    - tris (1,3-dichloro- 2- propyl) phosphate (TDCIPP),
    - tripropyl phosphate (TPP),
    - tri-n-butyl phosphate (TNBP),
    - 2-ethylhexyl diphenyl phosphate (EHDPP),
    - tricresyl phosphate (TMPP),
    - tripentyl phosphate (TPEP),
    - triphenyl phosphate (TPHP),
    - tris(2-ethylhexyl) phosphate (TEHP);
- Polychlorinated biphenyls (PCBs);
- Organochlorine pesticides (OCs):
  - hexachlorocyclohexanes (HCHs),
  - hexachlorobenzene (HCB),
  - chlordanes (CHLs),
  - dichlorodiphenyltrichloroethane (DDT);
- Toxic metals:
  - lead (Pb);
  - cadmium (Cd).

Despite their ubiquity in the environment, and the numerous reports on the levels of the aforementioned contaminants in food and dust samples from different countries, for Portugal there is limited or no information about their levels in these type of samples, and therefore the risks towards human health from exposure to these contaminants are unknown.

With this work, both pathways of exposure (ingestion of food and house dust) were characterized by evaluating the levels of FRs, PCBs, OCs and toxic metals in duplicate diet samples (representative of the real diet) and/or in house dust samples provided by

Portuguese adult citizens. Regarding the dietary intake, a duplicate diet study was implemented and volunteers were recruited from the academic community of the University of Aveiro (Portugal). For the intake through the ingestion of house dust, the dust samples were collected in houses from three Portuguese cities, namely Aveiro, Coimbra and Covilhã.

Besides the characterization of the levels of the target compounds in food and dust samples, the daily/weekly intakes of the contaminants were estimated for each participant and each pathway of exposure, and compared to the established tolerable daily/weekly intakes (TDI/TWI) or reference doses (RfD) in order to assess the associated risks to the exposed population.

1.6. Organization of the thesis

The present thesis is organized in four chapters. The first one provides an overview on the environmental contamination by chemicals resulting predominantly from anthropogenic activities; on the target organic and inorganic compounds; and the main pathways of human exposure. Chapter 2 comprises 4 subchapters structured as scientific papers describing the diet habits of a Portuguese academic community and assessing the dietary exposure to the target compounds. Chapter 3 comprises 3 subchapters and follows the same structure, characterizing the exposure through the ingestion of house dust. The fourth chapter provides a general discussion of the obtained results.

Chapter 1: provides an overview about the human exposure to environmental contaminants over the present century. It describes the main classes of contaminants and provides a brief introduction regarding the occurrence of the target compounds. This chapter also describes the major sources of human exposure.

Chapter 2: addresses the dietary exposure to environmental contaminants.

2.1: characterizes the dietary habits of a Portuguese academic community from the University of Aveiro, through the implementation of a food frequency questionnaire. This chapter is based on the submitted manuscript: *Coelho S.D., Maricoto T., Tanabe*

S., Sousa A.C.A., Nogueira A.J.A., (submitted). Dietary habits of a Portuguese academic community – a Food Frequency Questionnaire approach. Alimentação Humana.

2.2: describes the concentrations of brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) in duplicate diet samples provided by a Portuguese academic community (adults working or studying in the University of Aveiro, Portugal), and estimates the participants dietary intake of BFRs, PCBs and OCs. This chapter is based on the published paper: *Coelho S.D., Sousa A.C.A., Isobe T., Kunisue T., Nogueira A.J.A., Tanabe S., (2016). Brominated flame retardants and organochlorine compounds in duplicate diet samples from a Portuguese academic community. Chemosphere. 160: 89-94.*

2.3: describes the concentrations of lead (Pb) in duplicate diet samples provided by adults from a Portuguese academic community (working or studying in the University of Aveiro, Portugal), and characterizes the risk of cardiovascular and nephrotoxic effects associated with the ingestion of Pb through the Margin of Exposure (MOE) approach described by the European Food Safety Authority. This chapter is based on the published paper: *Coelho S.D., Pastorinho M.R., Itai T., Isobe T., Kunisue T., Nogueira A.J.A., Tanabe S., Sousa A.C.A., (2016). Lead in duplicate diet samples from an academic community. Science of the Total Environment. 573: 603-607.*

2.4: describes the concentrations of cadmium (Cd) in duplicate diet samples provided by premenopausal women from the University of Aveiro, and estimates the participants' dietary intake of Cd. This chapter is based on the submitted manuscript: *Coelho S.D., Maricoto T., Pastorinho M.R., Itai T., Isobe T., Kunisue T., Tanabe S., Sousa A.C.A., Nogueira A.J.A., (submitted). Cadmium intake in women from the University of Aveiro, Portugal – a duplicate diet study. Journal of Geochemical Exploration.*

Chapter 3: addresses the exposure to environmental contaminants via the ingestion of house dust.

3.1: provides a critical review of the global occurrence of two brominated flame retardants - polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs) – in indoor dust, the human exposure via dust ingestion and their occurrence in the human body. This chapter is based on the published review paper: *Coelho, S.D., Sousa, A.C.A., Isobe, T., Tanabe, S., Nogueira, A.J.A., 2014. Flame retardants in indoor dust - A review on the levels of polybrominated diphenyl ethers and hexabromocyclododecanes. Current Organic Chemistry. 18 (17): 2218-2230.*

3.2: describes the levels of brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and organophosphorus flame retardants (PFRs) in house dust samples from two Portuguese cities (Aveiro and Coimbra), and addresses the risks associated with dust ingestion by estimating their intakes. This chapter is based on the published paper: *Coelho S.D., Sousa A.C.A., Isobe T., Kim J.W., Kunisue T., Nogueira A.J.A., Tanabe S., 2016. Brominated, chlorinated and phosphate organic contaminants in house dust from Portugal. Science of the Total Environment.569-570: 442-449.*

3.3: compares the levels of brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) in house dust samples provided by non- asthmatic and asthmatic adults from Covilhã, Portugal, as well as their intakes, and describes the association of the detected levels and the incidence of asthma.

Chapter 4: provides a general discussion of the results obtained in chapter 2 and 3. Since each subchapter includes its specific discussion, in this section a global and brief discussion of all the obtained results is provided alongside with the most important conclusions obtained in this thesis.

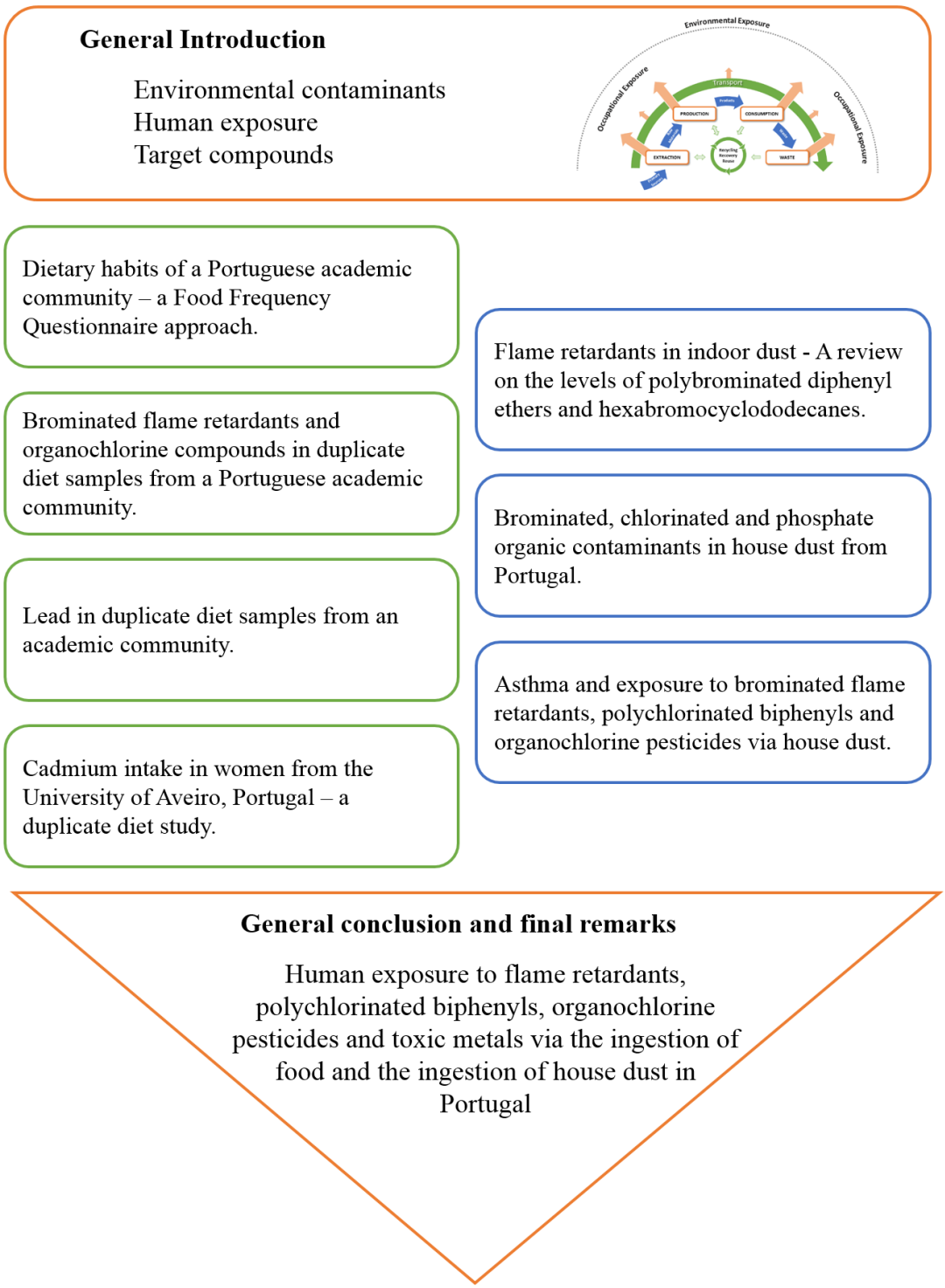

Figure 1.6.1. Illustrative scheme of the thesis organization.

References

Ali, M.H.H., Al-Qahtani, K.M., 2012. Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. Egypt. J. Aquat. Res. 38, 31-37.

ATSDR, 2015. The priority list of hazardous substances that will be the candidates for toxicological profiles. Agency for Toxic Substances and Disease Registry, Atlanta, GA. https://www.atsdr.cdc.gov/spl/ (accessed April, 2016).

Babrauskas, V., Fuoco, R., Blum, A., 2014. Chapter 3 - Flame Retardant Additives in Polymers: When do the Fire Safety Benefits Outweigh the Toxicity Risks? . in: Kiliaris, P. (Ed.). Polymer Green Flame Retardants. Elsevier, Amsterdam, pp. 87-118.

Becker, W., Jorhem, L., Sundström, B., Grawé, K.P., 2011. Contents of mineral elements in Swedish market basket diets. J. Food Compos. Anal. 24, 279-287.

Becking, G.C., Nordberg, M., Nordberg, G.F., 2007. CHAPTER 9 - Essential Metals: Assessing Risks from Deficiency and Toxicity. in: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.). Handbook on the Toxicology of Metals (Third Edition). Academic Press, Burlington, pp. 163-176.

Birnbaum, L.S., Staskal, D.F., 2004. Brominated flame retardants: Cause for concern? Environ. Health Persp. 112, 9-17.

Blum, A., 2007. The Fire Retardant Dilemma. Science 318, 194-195.

Blum, A., Ames, B., 1977. Flame-retardant additives as possible cancer hazards. Science 195, 17-23.

Blum, A., Gold, M.D., Ames, B.N., Jones, F.R., Hett, E.A., Dougherty, R.C., Horning, E.C., Dzidic, I., Carroll, D.I., Stillwell, R.N., Thenot, J.P., 1978. Children absorb tris-BP flame retardant from sleepwear: urine contains the mutagenic metabolite, 2,3-dibromopropanol. Science 201, 1020-1023.

Bortey-Sam, N., Nakayama, S.M.M., Ikenaka, Y., Akoto, O., Baidoo, E., Yohannes, Y.B., Mizukawa, H., Ishizuka, M., 2015. Human health risks from metals and metalloid via consumption of food animals near gold mines in Tarkwa, Ghana: Estimation of the daily intakes and target hazard quotients (THQs). Ecotox. Environ. Safe. 111, 160-167.

Brandsma, S.H., de Boer, J., van Velzen, M.J.M., Leonards, P.E.G., 2014. Organophosphorus flame retardants (PFRs) and plasticizers in house and car dust and the influence of electronic equipment. Chemosphere 116, 3-9.

Cairns, T., Siegmund, E.G., 1981. PCBs. Regulatory history and analytical problems. Anal. Chem. 53, 1183A-1193A.

Carson, R., 1962. Silent Spring. Mariner Books, Boston: Houghton Mifflin.

Casals-Casas, C., Desvergne, B., 2011. Endocrine Disruptors: From Endocrine to Metabolic Disruption. Annu. Rev. Physiol. 73, 135-162.

Cequier, E., Sakhi, A.K., Marcé, R.M., Becher, G., Thomsen, C., 2015. Human exposure pathways to organophosphate triesters — A biomonitoring study of mother–child pairs. Environ. Int. 75, 159-165.

Chen, D., Letcher, R.J., Chu, S., 2012. Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography–tandem quadrupole mass spectrometry. J. Chromatogr. A 1220, 169-174.

Coelho, S.D., Sousa, A.C.A., Isobe, T., Tanabe, S., Nogueira, A.J.A., 2014. Flame retardants in indoor dust - A review on the levels of polybrominated diphenyl ethers and hexabromocyclododecanes. Curr. Org. Chem. 18, 2218-2230.

Connolly, L., 2009. 5 - Endocrine-disrupting chemicals: origins, fates and transmission into the food chain. in: Shaw, I. (Ed.). Endocrine-Disrupting Chemicals in Food. Woodhead Publishing, pp. 103-125.

Cornelis, R., Nordberg, M., 2007. Chapter 2 - General Chemistry, Sampling, Analytical Methods, and Speciation*. in: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.). Handbook on the Toxicology of Metals (Third Edition). Academic Press, Burlington, pp. 11-38.

Covaci, A., Harrad, S., Abdallah, M.A.E., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532-556.

Danzer, A.M., Danzer, N., 2016. The long-run consequences of Chernobyl: Evidence on subjective well-being, mental health and welfare. J. Public Econ. 135, 47-60.

Domingo, J.L., 2012. Polybrominated diphenyl ethers in food and human dietary exposure: A review of the recent scientific literature. Food Chem. Toxicol. 50, 238-249.

Driffield, M., Harmer, N., Bradley, E., Fernandes, A.R., Rose, M., Mortimer, D., Dicks, P., 2008. Determination of brominated flame retardants in food by LC-MS/MS: diastereoisomer-specific hexabromocyclododecane and tetrabromobisphenol A. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk. Assess. 25, 895-903.

EEA, 2010. Pharmaceuticals in the environment: Results of an EEA workshop. in: Agency, E.E. (Ed.). EEA Technical report series 1725-2237, Copenhagen, p. 34.

EFSA, 2005. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the presence of non dioxin-like polychlorinated biphenyls (pcb) in feed and food. EFSA J. 1 - 137.

EFSA, 2010. Scientific Opinion on Lead in Food. EFSA J. 151.

EFSA, 2011. Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA J. 274.

EFSA, 2012. Cadmium dietary exposure in the European population. EFSA J. 37.

EFSA, 2014. A systematic procedure for the identification of emerging chemical risks in the food and feed chain. EFSA supporting publication. 40.

Endocrine Society, 2015. Endocrine-Disrupting Chemicals In The European Union - Position Statement. endocrine.org, Washington, pp. 4.

Fernandes, A.R., Mortimer, D., Rose, M., Smith, F., Panton, S., Garcia-Lopez, M., 2016. Bromine content and brominated flame retardants in food and animal feed from the UK. Chemosphere 150, 472-478.

Frederiksen, M., Thomsen, C., Frøshaug, M., Vorkamp, K., Thomsen, M., Becher, G., Knudsen, L.E., 2010. Polybrominated diphenyl ethers in paired samples of maternal and umbilical cord blood plasma and associations with house dust in a Danish cohort. Int. J. Hyg. Environ. Health 213, 233-242.

Fromme, H., Becher, G., Hilger, B., Völkel, W., 2015. Brominated flame retardants – Exposure and risk assessment for the general population. Int. J. Hyg. Environ. Health 219, 1-23.

Gilbert, S.G., 2011. Chapter 3 - A History of Toxicology. in: Healthy World Press (Ed.). A Small Dose of Toxicology, pp. 1-6.

Gold, M.D., Blum, A., Ames, B.N., 1978. Another flame retardant, tris-(1,3-dichloro-2-propyl)-phosphate, and its expected metabolites are mutagens. Science 200, 785-787.

Goyer, R., 2004. Issue paper on the human health effects of metals US-EPA, Washington, DC, pp. 48.

Grung, M., Lin, Y., Zhang, H., Steen, A.O., Huang, J., Zhang, G., Larssen, T., 2015. Pesticide levels and environmental risk in aquatic environments in China — A review. Environ. Int. 81, 87-97.

Gulson, B., Anderson, P., Taylor, A., 2013. Surface dust wipes are the best predictors of blood leads in young children with elevated blood lead levels. Environ. Res. 126, 171-178.

Halldorsson, T.I., 2012. Dietary Exposures to Persistent Organic Pollutants and Fetal Growth. in: Preedy, R.V. (Ed.). Handbook of Growth and Growth Monitoring in Health and Disease. Springer New York, New York, NY, pp. 2559-2578.

Hinwood, A.L., Callan, A.C., Heyworth, J., Rogic, D., de Araujo, J., Crough, R., Mamahit, G., Piro, N., Yates, A., Stevenson, G., Odland, J.O., 2014. Polychlorinated biphenyl (PCB) and dioxin concentrations in residential dust of pregnant women. Environ. Sci. Process. Impacts 16, 2758-2763.

Hogervorst, J., Plusquin, M., Vangronsveld, J., Nawrot, T., Cuypers, A., Van Hecke, E., Roels, H.A., Carleer, R., Staessen, J.A., 2007. House dust as possible route of environmental exposure to cadmium and lead in the adult general population. Environ. Res. 103, 30-37.

Holden, A.V., 1970. Monitoring organochlorine contamination of the marine environment by the analysis of residues in seals. in: Ruivo, M. (Ed.). Marine Pollution and Sea Life. FAO, Fishing News Books, Surrey, pp. 266-272.

Holt, M.S., 2000. Sources of chemical contaminants and routes into the freshwater environment. Food Chem. Toxicol. 38, Supplement 1, S21-S27.

IARC, 1990. Chromium, nickel and welding. IARC monographs on the evaluation of carcinogenic risks to humans, pp. 648.

IARC, 2006. Inorganic and organic lead compounds. IARC Monographs on the evaluation of carcinogenic risks to human, pp. 519.

IARC, 2012. Cadmium and Cadmium Compounds. IARC monographs on the evaluation of carcinogenic risks to humans, pp. 26.

Ibanez, Y., Le Bot, B., Glorennec, P., 2010. House-dust metal content and bioaccessibility: a review. Eur. J. Mineral. 22, 629-637.

IPCS, 2004. IPCS Risk Assessment Terminology. International Programme on Chemical Safety, World Health Organization, Geneva, p. 122.

Islam, M.S., Ahmed, M.K., Habibullah-Al-Mamun, M., Raknuzzaman, M., 2015. The concentration, source and potential human health risk of heavy metals in the commonly consumed foods in Bangladesh. Ecotox. Environ. Safe. 122, 462-469.

Iwata, H., Tanabe, S., Sakai, N., Tatsukawa, R., 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. Environ. Sci. Technol. 27, 1080-1098.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B., Beeregowda, K.N., 2014. Toxicity, mechanism and health effects of some heavy metals. Interdiscip. Toxicol. 7, 60-72.

Jakobsson, K., Thuresson, K., Rylander, L., Sjodin, A., Hagmar, L., Bergman, A., 2002. Exposure to polybrominated diphenyl ethers and tetrabromobisphenol A among computer technicians. Chemosphere 46, 709-716.

Jensen, S., Johnels, A.G., Olsson, M., Otterlind, G., 1969. DDT and PCB in Marine Animals from Swedish Waters. Nature 224, 247-250.

Johnson, P.I., Stapleton, H.M., Mukherjee, B., Hauser, R., Meeker, J.D., 2013. Associations between brominated flame retardants in house dust and hormone levels in men. Sci. Total Environ. 445-446, 177-184.

Kabir, E.R., Rahman, M.S., Rahman, I., 2015. A review on endocrine disruptors and their possible impacts on human health. Environ. Toxicol. Phar. 40, 241-258.

Kalachova, K., Hradkova, P., Lankova, D., Hajslova, J., Pulkrabova, J., 2012. Occurrence of brominated flame retardants in household and car dust from the Czech Republic. Sci. Total Environ. 441, 182-193.

Kannan, K., Tanabe, S., Giesy, J.P., Tatsukawa, R., 1997. Organochlorine pesticides and polychlorinated biphenyls in foodstuffs from Asian and oceanic countries. Rev. Environ. Contam. Toxicol. 152, 1-55.

Karlsson, M., Julander, A., van Bavel, B., Hardell, L., 2007. Levels of brominated flame retardants in blood in relation to levels in household air and dust. Environ. Int. 33, 62-69.

Kim, J.-W., Isobe, T., Chang, K.-H., Amano, A., Maneja, R.H., Zamora, P.B., Siringan, F.P., Tanabe, S., 2011. Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines. Environ. Pollut. 159, 3653-3659.

Koch, W., Karim, M.R., Marzec, Z., Miyataka, H., Himeno, S., Asakawa, Y., 2016. Dietary intake of metals by the young adult population of Eastern Poland: Results from a market basket study. J. Trace Elem. Med. Bio. 35, 36-42.

Kucharska, A., Cequier, E., Thomsen, C., Becher, G., Covaci, A., Voorspoels, S., 2015. Assessment of human hair as an indicator of exposure to organophosphate flame retardants. Case study on a Norwegian mother-child cohort. Environ. Int. 83, 50-57.

Le Cann, P., Bonvallot, N., Glorennec, P., Deguen, S., Goeury, C., Le Bot, B., 2011. Indoor environment and children's health: Recent developments in chemical, biological, physical and social aspects. Int. J. Hyg. Environ. Health 215, 1-18.

Li, Q.Q., Loganath, A., Chong, Y.S., Tan, J., Obbard, J.P., 2006. Persistent organic pollutants and adverse health effects in humans. J. Toxicol. Environ. Health A 69, 1987-2005.

Lioy, P.J., 1990. Assessing total human exposure to contaminants. A multidisciplinary approach. Environ. Sci. Technol. 24, 938-945.

Loutfy, N., Fuerhacker, M., Tundo, P., Raccanelli, S., El Dien, A.G., Ahmed, M.T., 2006. Dietary intake of dioxins and dioxin-like PCBs, due to the consumption of dairy products, fish/seafood and meat from Ismailia city, Egypt. Sci. Total Environ. 370, 1-8.

Macdonald, R.W., Harner, T., Fyfe, J., 2005. Recent climate change in the Arctic and its impact on contaminant pathways and interpretation of temporal trend data. Sci. Total Environ. 342, 5-86.

Malisch, R., Kotz, A., 2014. Dioxins and PCBs in feed and food - Review from European perspective. Sci. Total Environ.

Mansour, S.A., 2011. Chemical pollutants threatening food safety and security: An overview. pp. 73-117.

Martellini, T., Diletti, G., Scortichini, G., Lolini, M., Lanciotti, E., Katsoyiannis, A., Cincinelli, A., 2016. Occurrence of polybrominated diphenyl ethers (PBDEs) in foodstuffs in Italy and implications for human exposure. Food Chem. Toxicol. 89, 32-38.

Martorell, I., Perello, G., Marti-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2011. Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend. Biol. Trace Elem. Res. 142, 309-322.

Meador, J.P., Yeh, A., Young, G., Gallagher, E.P., 2016. Contaminants of emerging concern in a large temperate estuary. Environ. Pollut. 213, 254-267.

Mercier, F., Glorennec, P., Thomas, O., Bot, B.L., 2011. Organic contamination of settled house dust, a review for exposure assessment purposes. Environ. Sci. Technol. 45, 6716-6727.

Mizouchi, S., Ichiba, M., Takigami, H., Kajiwara, N., Takamuku, T., Miyajima, T., Kodama, H., Someya, T., Ueno, D., 2015. Exposure assessment of organophosphorus and organobromine flame retardants via indoor dust from elementary schools and domestic houses. Chemosphere 123, 17-25.

Mohammed, A.S., Kapri, A., Goel, R., 2011. Heavy Metal Pollution: Source, Impact, and Remedies. in: Khan, S.M., Zaidi, A., Goel, R., Musarrat, J. (Eds.). Biomanagement of Metal-Contaminated Soils. Springer Netherlands, Dordrecht, pp. 1-28.

Moya, J., Phillips, L., 2014. A review of soil and dust ingestion studies for children. J. Expos. Sci. Environ. Epidemiol. 24, 545-554.

Naja, G., Volesky, B., 2009. Chapter 2 - Toxicity and sources of Pb, Cd, Hg, Cr, As, and radionuclides in the environment. in: Wang, L., Chen, J., Hung, Y., Shammas, N. (Eds.). Heavy metals in the environment. CRC Press LCL, Boca, Raton, pp. 13-61.

Nasreddine, L., Nashalian, O., Naja, F., Itani, L., Parent-Massin, D., Nabhani-Zeidan, M., Hwalla, N., 2010. Dietary exposure to essential and toxic trace elements from a Total diet study in an adult Lebanese urban population. Food Chem. Toxicol. 48, 1262-1269.

Newton, S., Sellström, U., de Wit, C.A., 2015. Emerging Flame Retardants, PBDEs, and HBCDDs in indoor and outdoor media in Stockholm, Sweden. Environ. Sci. Technol. 49, 2912-2920.

Noonan, C.W., Kathman, S.J., Sarasua, S.M., White, M.C., 2003. Influence of environmental zinc on the association between environmental and biological measures of lead in children. J. Expo. Anal. Environ. Epidemiol. 13, 318-323.

Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T., 2007a. Chapter 1 - Introduction—General Considerations and International Perspectives. in: Nordberg, G.F., Fowler, B.A.,

Nordberg, M., Friberg, L.T. (Eds.). Handbook on the Toxicology of Metals (Third Edition). Academic Press, Burlington, pp. 1-9.

Nordberg, G.F., Nogawa, K., Nordberg, M., Friberg, L.T., 2007b. Chapter 23 - Cadmium. in: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.). Handbook on the Toxicology of Metals (Third Edition). Academic Press, Burlington, pp. 445-486.

Norstrom, R.J., Muir, D.C., 1994. Chlorinated hydrocarbon contaminants in arctic marine mammals. Sci. Total Environ. 154, 107-128.

Norton, G.J., Deacon, C.M., Mestrot, A., Feldmann, J., Jenkins, P., Baskaran, C., Meharg, A.A., 2015. Cadmium and lead in vegetable and fruit produce selected from specific regional areas of the UK. Sci. Total Environ. 533, 520-527.

Pavón, J.M.C., de Torres, A.G., Rojas, F.S., Ojeda, C.B., Alonso, E.V., 2015. The toxic elements. in: de la Guardia, M., Garrigues, S. (Eds.). Handbook of Mineral Elements in Food. John Wiley & Sons, Ltd, pp. 123-152.

Prüss-Ustün, A., Vickers, C., Haefliger, P., Bertollini, R., 2011. Knowns and unknowns on burden of disease due to chemicals: a systematic review. Environ. Health 10, 1-15.

Quintana, P.J., Mohammadian, M., Matt, G.E., 2008. Heavy Metal Contamination of House Dust in Homes of Mothers that Smoke in Proximity to Their Infant. Epidemiology 19, S214.

Raghav, M., Eden, S., Mitchell, K., Witte, B., 2013. Arroyo 2013 Contaminants of Emerging Concern in Water. Water Resources Research Center, Tucson, Arizona, pp. 12.

Raven, P.H., Hassenzahl, D.M., Berg, L.R., 2012. Environment, 8th Edition. John Wiley & Sons.

REACH, 2006. Environment fact sheet: REACH a new chemicals policy for the EU. European Commission pp. 1-4.

Roosens, L., Abdallah, M.A.E., Harrad, S., Neels, H., Covaci, A., 2009. Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: Preliminary results. Environ. Health Persp. 117, 1707-1712.

Ruder, A.M., Hein, M.J., Hopf, N.B., Waters, M.A., 2014. Mortality among 24,865 workers exposed to polychlorinated biphenyls (PCBs) in three electrical capacitor manufacturing plants: A ten-year update. Int. J. Hyg. Environ. Health 217, 176-187.

Saravi, S.S.S., Dehpour, A.R., 2016. Potential role of organochlorine pesticides in the pathogenesis of neurodevelopmental, neurodegenerative, and neurobehavioral disorders: A review. Life Sci. 145, 255-264.

Sauvé, S., Desrosiers, M., 2014. A review of what is an emerging contaminant. Chem. Cent. J. 8, 15.

Schecter, A., Papke, O., Harris, T.R., Tung, K.C., Musumba, A., Olson, J., Birnbaum, L., 2006. Polybrominated diphenyl ether (PBDE) levels in an expanded market basket survey of U.S.

food and estimated PBDE dietary intake by age and sex. Environ. Health Perspect. 114, 1515-1520.

Shaw, S.D., Berger, M.L., Harris, J.H., Yun, S.H., Wu, Q., Liao, C., Blum, A., Stefani, A., Kannan, K., 2013. Persistent organic pollutants including polychlorinated and polybrominated dibenzo-p-dioxins and dibenzofurans in firefighters from Northern California. Chemosphere 91, 1386-1394.

Singh, R., Gautam, N., Mishra, A., Gupta, R., 2011. Heavy metals and living systems: An overview. Indian J. Pharmacol. 43, 246-253.

Singleton, B., Turner, J., Walter, L., Lathan, N., Thorpe, D., Ogbevoen, P., Daye, J., Alcorn, D., Wilson, S., Semien, J., Richard, T., Johnson, T., McCabe, K., Estrada, J.J., Galvez, F., Velasco, C., Reiss, K., 2016. Environmental stress in the Gulf of Mexico and its potential impact on public health. Environ. Res. 146, 108-115.

Sjodin, A., Hagmar, L., Klasson-Wehler, E., Kronholm-Diab, K., Jakobsson, E., Bergman, A., 1999. Flame retardant exposure: polybrominated diphenyl ethers in blood from Swedish workers. Environ. Health Perspect. 107, 643-648.

Skerfving, S., Bergdahl, I.A., 2007. Chapter 31 - Lead. in: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.). Handbook on the Toxicology of Metals (Third Edition). Academic Press, Burlington, pp. 599-643.

Sousa, A.C.A., Pastorinho, M.R., Takahashi, S., Tanabe, S., 2013. Organotin Compounds from Snails to Humans. in: Lichtfouse, E., Schwarzbauer, J., Robert, D. (Eds.). Pollutant Diseases, Remediation and Recycling. Springer International Publishing, pp. 215-275.

Stapleton, H.M., Eagle, S., Sjödin, A., Webster, T.F., 2012. Serum PBDEs in a North Carolina toddler cohort: Associations with handwipes, house dust, and socioeconomic variables. Environ. Health Persp. 120, 1049-1054.

Stapleton, H.M., Sjodin, A., Jones, R.S., Niehuser, S., Zhang, Y., Patterson, D.G., Jr., 2008. Serum levels of polybrominated diphenyl ethers (PBDEs) in foam recyclers and carpet installers working in the United States. Environ. Sci. Technol. 42, 3453-3458.

Stockholm Convention, 2015. Listing of POPs in the Stockholm Convention. http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx (accessed April, 2016)

Szirmai, A., 2012. Industrialisation as an engine of growth in developing countries, 1950–2005. Struct. Change Econ. Dynam. 23, 406-420.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy Metals Toxicity and the Environment. EXS 101, 133-164.

Tilley, S.K., Fry, R.C., 2015. Chapter 6 - Priority Environmental Contaminants: Understanding Their Sources of Exposure, Biological Mechanisms, and Impacts on Health. in: Fry, R.C. (Ed.). Systems Biology in Toxicology and Environmental Health. Academic Press, Boston, pp. 117-169.

UNEP, 2013. Global Chemicals Outlook - Towards sound management of chemicals. in: Kemf, E. (Ed.). United Nations Environment Programme, pp. 1-264.

US-EPA, 2010. Final review of scientific information on lead –Version of December 2010 United Nations Environment Programme. Chemicals Branch, DTIE, pp. 332.

US-EPA, 2011. Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC.

US-EPA, 2014. Technical Fact Sheet – Polybrominated Diphenyl Ethers (PBDEs) and Polybrominated Biphenyls (PBBs). pp. 7.

US-EPA, 2015a. Exposure Assessment Tools by Routes. https://www.epa.gov/expobox/exposure-assessment-tools-routes. (accessed April, 2016).

US-EPA, 2015b. Flame retardants in printed circuit boards - Chapter 3. EPA Publication, pp. 13.

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119-1153.

Vandermeersch, G., Lourenço, H.M., Alvarez-Muñoz, D., Cunha, S., Diogène, J., Cano-Sancho, G., Sloth, J.J., Kwadijk, C., Barcelo, D., Allegaert, W., Bekaert, K., Fernandes, J.O., Marques, A., Robbens, J., 2015. Environmental contaminants of emerging concern in seafood – European database on contaminant levels. Environ. Res. 143, Part B, 29-45.

Voorspoels, S., Covaci, A., Neels, H., Schepens, P., 2007. Dietary PBDE intake: a market-basket study in Belgium. Environ. Int. 33, 93-97.

Wania, F., MacKay, D., 1996. Peer Reviewed: Tracking the Distribution of Persistent Organic Pollutants. Environ. Sci. Technol. 30, 390A-396A.

Whitehead, T., Metayer, C., Buffler, P., Rappaport, S.M., 2011. Estimating exposures to indoor contaminants using residential dust. J. Expos. Sci. Environ. Epidemiol. 21, 549-564.

WHO, 2010. Exposure to cadmium: A major public health concern. Public Health and Environment - World Health Organization, pp. 4.

WHO/UNEP, 2013. State of the Science of Endocrine Disrupting Chemicals 2012 - Summary for Decision-Makers. in: Bergman, Å., Heindel, J.J., Jobling, S., Kidd, K.A., Zoeller, R.T. (Eds.), pp.38.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., La Guardia, M., McClean, M.D., Webster, T.F., 2007. Human Exposure to PBDEs: Associations of PBDE Body

Burdens with Food Consumption and House Dust Concentrations. Environ. Sci. Technol. 41, 1584-1589.

Yamashita, S., Ohto, H., Abe, M., Tanigawa, K., Yamashita, S., Kamiya, K., Yasumura, S., Hosoya, M., Suzuki, S., Ohtsuru, A., Sakai, A., Yabe, H., Maeda, M., Fujimori, K., Ishikawa, T., Ohira, T., Watanabe, T., Satoh, H., Suzuki, S., Fukushima, T., Midorikawa, S., Shimura, H., Matsuzuka, T., Mashiko, H., Goto, A., Nollet, K., Takahashi, H., Shibata, Y., Miyazaki, M., Matsui, S., Tanaka, S., 2016. Comprehensive Health Risk Management after the Fukushima Nuclear Power Plant Accident. Clin. Oncol. 28, 255-262.

Yusà, V., Pardo, O., 2015. Human risk assessment and regulatory framework for minerals in food. in: de la Guardia, M., Garrigues, S. (Eds.). Handbook of Mineral Elements in Food, pp. 69-108.

Zhang, L., Ma, J., Tian, C., Li, Y., Hung, H., 2010. Atmospheric transport of persistent semi-volatile organic chemicals to the Arctic and cold condensation in the mid-troposphere – Part 2: 3-D modeling of episodic atmospheric transport. Atmos. Chem. Phys. 10, 7315–7324.

Zheng, J., Luo, X.-J., Yuan, J.-G., Wang, J., Wang, Y.-T., Chen, S.-J., Mai, B.-X., Yang, Z.-Y., 2011. Levels and sources of brominated flame retardants in human hair from urban, e-waste, and rural areas in South China. Environ. Pollut. 159, 3706-3713.

CHAPTER 2

DIET

2.1. Dietary habits of a Portuguese academic community – a Food Frequency Questionnaire approach

Abstract

In Portugal, the traditional diet is Mediterranean, however, and similarly to what is happening in other countries, the eating habits are changing, particularly among younger generations. As such, the aim of this study was to evaluate the dietary habits of a young and literate population. To the best of our knowledge, this is the first study on the diet of an academic population in Portugal through the implementation of a food frequency questionnaire. The questionnaire was developed and applied to a non-random sample of 219 individuals, selected by convenience, studying or working in the University of Aveiro, Portugal. It was structured in order to evaluate the consumption frequency of several food items pooled in 16 groups. Rating was performed through multiple choice answers attributed to five consumption frequency categories: never, occasionally, weekly, daily or more than once per day. The food category with the highest consumption rates was dairy products (90% of daily consumption), followed by other products such as several carbohydrates, vegetables and fruit, and olive oil as the favourite cooking oil. For 76.7% of the volunteers the body mass index was between the normal range (18.50 and 24.99 Kg/m^2). The consumption of the food items characteristic of the Mediterranean diet is notable. However, the dietary profile of this community does not strictly follow the recommendations for a healthy diet.

Keywords: food frequency questionnaire, academic community, diet, Portugal.

2.1.1. Introduction

Diet is determinant for the human health condition, thus, the quantity and quality of consumed food items are crucial for a healthy lifestyle. It integrates a significant part of our daily life, and for most people it is more than a survival matter. Eating is also a cultural and social act. In fact, the diet style is considered to be a consequence of several eating behaviours based in psychological and social factors (Poínhos et al., 2013). Therefore, the types of food items consumed may change according to the social status, lifestyle and professional activity. Diet also varies between countries. According to Oxford Committee for Famine Relief [1] (OXFAM, 2014), Portugal is among the 12 countries (mainly Western European countries and Australia) considered as the best ones to eat around the world. In the OXFAM study, food consumption habits were analysed in 125 countries based on the four core questions: "*Do people have enough to eat?*", "*Can people afford to eat?*", "*Is food of good quality?*" and "*What is the extent of unhealthy outcomes of people's diet?*". This prime position might be a consequence of the Mediterranean diet adopted in Portugal. Portuguese diet is typically composed by fresh, local and seasonal food (INE, 2014).

The information about the consumed food is crucial for the assessment of the risk and impact of different nutrients and toxic chemicals on consumers' health (Vilone et al., 2014). According to the World Health Organization (WHO), contamination of food products by harmful bacteria, viruses, parasites and toxic chemicals is responsible for more than 200 diseases ranging from a simple diarrhoea to chronic diseases such as cancer (World Health Organization, 2014a). It is, therefore, important to develop studies that allow the description of the type, quantity and quality of the consumed food items and to associate diet and health.

These studies are frequently complemented by Food Frequency Questionnaires (FFQ). FFQs are considered as one of the key tools in epidemiological studies of diet and health (Cade et al., 2004). They were established during the 80's and 90's and have been used since then to monitor the populations' food intake. This tool is one of the most commonly used methods in the assessment of food consumption and its association with several health problems, as the FFQ adapts itself to the characteristics of the studied population, it

[1] Oxfam is an international confederation of 17 organizations working in more than 90 countries to find solutions to poverty and injustice around the world.

is easy to perform and cost effective (Sauvageot et al., 2013; Maruyama et al., 2015). Despite the utility of this kind of questionnaire, the number of studies on the Portuguese population feeding habits, using this tool, is scarce (e.g. Lopes et al., 2006). However, studies about diet as well as its toxicological characterization are extremely important.

The present work is part of an ongoing pilot study about the characterization of environmental contaminants in the diet of an academic community (Coelho et al., 2014; Sousa et al., 2014), that started with the collection of food samples and the application of a FFQ. Considering that the consumed food can be associated with the type of diet adopted in each country, and with the social status and occupation of the individual, the aim of this work is to characterize and describe the type of food consumed by this academic community.

2.1.2. Material and Methods

2.1.2.1. Food Frequency Questionnaire

In order to describe and characterize the dietary habits of the University of Aveiro academic community, a food frequency questionnaire (FFQ) was developed. The FFQ was distributed through the University of Aveiro (UA) email list and was available online from May to September 2012. The non-random sample, selected by convenience, consisted of 219 individuals from the university including students, researchers, teaching and non-teaching staff, who voluntarily joined the study.

The questionnaire was structured in order to evaluate the consumption frequency of several food items pooled in 16 groups. These groups were classified as: fish (including canned fish); seafood; meat; eggs; vegetables; fruit; dairy products; soy and derivatives; bread, cereals and similar; oils and fats; sweets and pastry; deep-fried foods (croquettes, meat or fish patties, etc.); mayonnaise, ketchup and mustard; pre-cooked meals; fast food; and drinks (including canned drinks). The origin of several food products (home grown; organic; other) and water (public; water well/borehole; fountain; bottled; filtered) were also included. Rating was performed through multiple choice answers attributed to five consumption frequency categories: never, occasionally, weekly (from 1 to 6 times a week), daily or more than once per day. In order to analyse data as consumption percentage, a

score was assigned to each option and the two last options where pooled together as "daily" which means 7 or more times a week.

2.1.2.2. Data Analysis

Data analysis was performed using the software Statistical Package for Social Sciences (IBM SPSS Statistics 20). Results are presented as descriptive statistics.

2.1.3. Results

Two hundred and nineteen people from 18 to 49 years old answered the FFQ. From the obtained sample (characterized in Table 2.1.1), 169 (77.2%) volunteers were women, while 50 (22.8%) were men. Body Mass Index (BMI) was calculated for each participant considering the body weights and heights. For 76.7% of the volunteers the BMI was between 18.50 and 24.99 Kg/m^2 (normal range, set by the World Health Organization (WHO, 2006)). The mean BMI for women was 21.7 Kg/m^2 and for men it was 24.7 Kg/m^2. As the sample comprises an academic community, all participants accomplished the Portuguese compulsory education (high school). From this group, 141 (64.4%) were students (BSc, MSc and PhD, see Table 2.1.1); 23 (10.5%) and 22 (10%) were associate researchers and postdoctoral researchers, respectively; and 33 (15.1%) were employees, professors and others (Table 2.1.1).

Table 2.1.2 shows the results of the consumption frequency profile for each group of food, with higher prevalence of food classes such as fish, vegetables, fruit, meat, dairy products, olive oil and most basic carbohydrates.

The food category with the highest consumption rates was dairy products (90% of daily consumption), mostly due to the high daily consumption of yogurt (60.2%) and low fat milk (52.1%). Daily consumption of carbohydrates (85.8%), vegetables (80.4%) and fruits (67.1%) was also high (Figure 2.1.1 and Table 2.1.2).

Table 2.1.1. Population characteristics.

	No. of participants	% of participants
Gender		
Women	169	77.2
Men	50	22.8
Age (years)		
<20	9	4.1
20-24	83	37.9
25-29	68	31.1
30-34	30	13.7
35-39	18	8.2
40-44	7	3.2
45-49	4	1.8
BMI* (kg/m^2)		
<18.50	11	5.0
18.50 - 24.99	168	76.7
≥25.00	40	18.3
Professional Status		
BSc student	46	21.0
MSc student	51	23.3
PhD student	43	19.6
Non - teaching staff	9	4.1
Teaching staff	8	3.7
Researcher	23	10.5
Postdoctoral researcher	22	10.0
Others	16	7.3

***Body mass index ranges set by World Health Organization (2006):
<18.50: Underweight
18.50 - 24.99: Normal range
≥25.00 : Overweight

Generally, meat was consumed more frequently (27.9% daily) than fish (4.6% daily) (Table 2.1.2). Processed meats such as ham, sausage and salami were the most consumed meat type on a daily basis (18.7%) followed by chicken (10.5%) (Figure 2.1.1).

Processed food, which includes deep-fried food, pre-cooked meals and fast food, is not frequently consumed by the participants (Figure 2.1.1), except on an occasional basis, 63.9%, 61.2% and 80.8%, respectively (Table 2.1.2).

As for canned food (fish, vegetables, fruit, olives and drinks), the weekly consumption frequency was 66.2% (62.7% in the case of women and 78.0% for men). The most frequently consumed canned food was fish (47.5%), followed by vegetables (32.4%), olives (22.4%), drinks (16.4%) and fruits (6.4%).

Generally, oils and fats were frequently consumed (83.1% daily). The favourite cooking oil was olive oil, used daily by 79.5% of the participants. Other important characteristic in this population's feeding profile is the daily consumption of sources of fast absorption sugar such as sweets and pastry (68.0%); refined sugar (44.7%) and cookies (including "Maria cookies", water cookies and whole-wheat crackers - 22%).

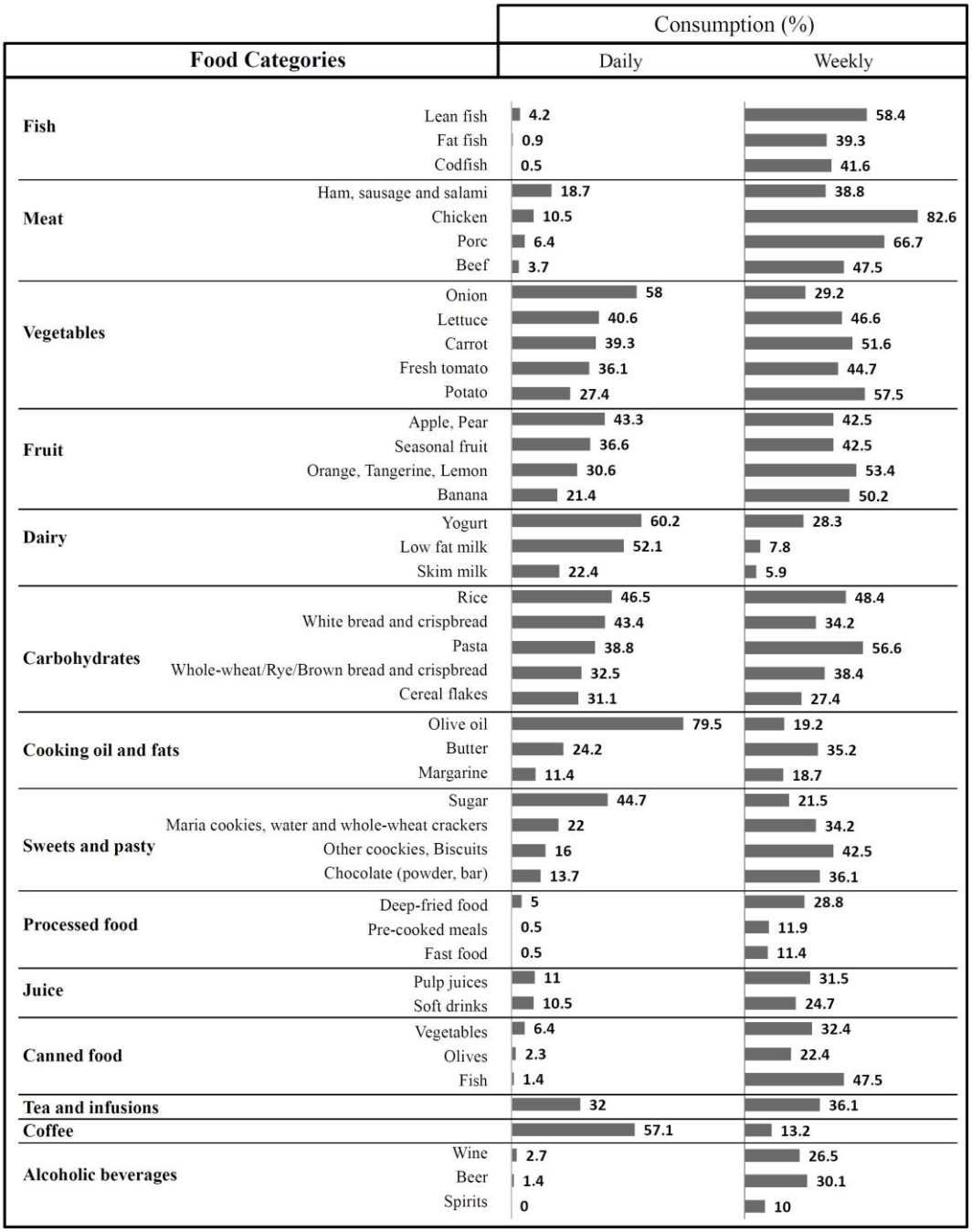

Figure 2.1.1. Most consumed food items, daily and weekly, in selected categories.

Table 2.1.2. Feeding frequency profile considering each category of food.

Food Categories	Daily consumption Total % (Women %; Men %)	Weekly consumption Total % (Women %; Men %)	Occasional consumption Total % (Women %; Men %)	No consumption Total % (Women %; Men %)
Dairy products	90.0 (91.7; 84.0)	6.8 (5.9; 10.0)	2.7 (1.8; 6.0)	0.5 (0.6; 0)
Carbohydrates	85.8 (87.6; 80.0)	13.2 (11.2; 20.0)	0.5 (0.6; 0)	0.5 (0.6; 0)
Vegetables	80.4 (82.2; 74.0)	19.2 (17.2;26.0)	0.5 (0.6; 0)	0
Fruit	67.1 (71.0; 54.0)	30.1 (26.0; 44.0)	2.7 (3.0; 2.0)	0
Meat	27.9 (24.3; 40.0)	68.9 (72.8; 56.0)	1.4 (1.2; 2.0)	1.8 (1.8; 2.0)
Fish	4.6 (4.7; 4.0)	80.8 (82.8; 74.0)	12.8 (10.7; 20.0)	1.8 (1.8; 2.0)
Seafood	0	4.1 (3.0; 8.0)	81.7 (81.7; 82.0)	14.2 (15.4; 10.0)
Eggs	1.4 (1.2; 2.0)	71.7 (71.6; 72.0)	26.0 (26.0; 26.0)	0.9 (1.2; 0)
Soy and derivatives	3.7 (4.7; 0)	12.8 (12.4;14.0)	40.6 (39.6; 44.0)	42.9 (43.2; 42.0)
Deep-fried foods (French fries, croquettes, meat or fish patties, etc.)	5.0 (3.0; 12.0)	28.8 (25.4; 40.0)	63.9 (68.6; 48.0)	2.3 (3.0; 0)
Pre-cooked meals	0.5 (0.6; 0.5)	11.9 (9.5; 20.0)	61.2 (62.1; 58.0)	26.5 (27.8; 22.0)
Fast food	0.5 (0.6; 0)	11.4 (8.9; 20.0)	80.8 (84.0; 70.0)	7.3 (6.5; 10.0)
Canned food	10.5 (9.5; 14.0)	66.2 (62.7; 78.0)	22.8 (27.2; 8.0)	0.5 (0.6; 0)
Oils and fats	83.1 (82.8; 84.0)	15.5 (16.0; 14.0)	1.4 (1.2; 2.0)	0
Mayonnaise, ketchup, mustard, etc.	0.9 (0.6; 2.0)	15.5 (13.6; 22.0)	68.9 (69.8; 66.0)	14.6 (16.0; 10.0)
Sweets and pastry	68.0 (75.7; 42.0)	25.6 (20.1; 44.0)	5.9 (4.1; 12.0)	0.5 (0; 2.0)
Pulp juices and soft drinks	15.5 (14.2; 20.0)	37.9 (34.9; 48.0)	42.5 (46.2; 30.0)	4.1 (4.7; 2.0)
Coffee	57.1 (55.0; 64.0)	13.2 (14.8; 8.0)	12.3 (11.2; 16.0)	17.4 (18.9; 12.0)
Tea and infusions	32.0 (36.1; 18.0)	36.1 (36.7; 34.0)	26.9 (21.3; 46.0)	5.0 (5.9; 2.0)
Alcoholic beverages	4.1 (3.6; 6.0)	39.3 (32.0; 64.0)	42.9 (47.9; 26.0)	13.7 (16.6; 4.0)

It is also important to mention the weekly consumption frequency of juices (37.9%), such as pulp juices (31.5%) and soft drinks (24.7%).

Coffee and tea are an integral part of the Portuguese diet and therefore there is a relative high frequency of daily consumption (57.1 and 32.0%, respectively).

The number of participants consuming alcoholic beverages on a weekly basis (39.3%) is also considerable despite the relatively low number that consumed daily (4.1%). Men's alcohol weekly consumption (64.0%) is twice that of women (32.0%).

Concerning the origin of food, the consumption of organic products was low varying from 6.8 to 10.5%, being the organic fruit the most consumed (10.5%). Considering the home grown food (consumption ranging from 2.7 to 48.4%), eggs were on top of the list, second place went for vegetables (29.7%) followed by meat (18.3%). As for water, bottled water was the most commonly consumed (63.5%) followed by public water (19.6%).

2.1.4. Discussion

The aim of this work was to describe the diet of an academic community from the University of Aveiro, Portugal. This sample was composed by 169 women and 50 men with ages from 18 to 49 years old and exhibited a feeding profile close to the typical Mediterranean diet which privileges fresh, local and seasonal food products, mainly olive oil, cereals, fruits and vegetables and moderate amounts of fish, dairy products, meat and wine (Petrillo, 2012).

According to WHO, the normal values of BMI for adults range from 18.50 to 24.99 Kg/m^2 (World Health Organization, 2006). Although 18.3% of the studied population showed BMI values equal or greater than 25 Kg/m^2, suggesting overweight (Table 2.1.1), generally it exhibited a BMI profile considered normal with mean values of 21.7 Kg/m^2 in the case of women and 24.7 Kg/m^2 for men. The participants' BMI mean values are lower than those estimated by WHO for Portugal in 2010 (25.7 and 26.5 Kg/m^2 in women and men, respectively) and 2014 (25.7 and 26.7 Kg/m^2 in women and men, respectively) (World Health Organization, 2014c). These divergent results might be a consequence of the group socio-economic characteristics. Probably, the high educational level led to an advanced knowledge on healthy diet and lifestyle habits. This can also explain the percentage of

persons (63.0%) which attempt to consume home grown food items or organic food (especially eggs, vegetables and fruit).

As shown in Figure 2.1.1 and Table 2.1.2, the consumption of the food items characteristic of the Mediterranean diet is notable. High daily frequencies can be observed for dairy products, several carbohydrates, vegetables and fruit, and olive oil as the favourite cooking oil.

Considering the consumption of fish and meat, it is possible to notice a preference towards meat, particularly chicken. This tendency was previously described in the report of the Portuguese Food Balance in 2008-2012 published by the National Institute of Statistics Portugal which disclosed a progressive increase in the demand of poultry meat and a decrease in the availability of beef and pork in recent years (INE, 2014).

A similar FFQ study, the EpiPorto, was performed in a large population from the Oporto city, Portugal in 2006 (Lopes et al., 2006), with ages ranging from 18 to more than 65 years old. When comparing both studies the frequencies of consumption of dairy products, carbohydrates, vegetables and fruits were similar. However, some differences appear when considering the fish and meat consumption: with daily intakes of 4.6% for fish and 27.9% for meat in our study, and 31.2 - 33.6% for fish and 51 - 63.4% for meat in the EpiPorto survey. Daily consumption of oils and fats was higher in our study (83.1% vs 60.2 - 69.2%) as well as the weekly consumption of pulp juices and soft drinks (37.9% vs 13.8 – 16.0%). Such differences might be explained by the fact that our study was performed in 2012 and EpiPorto six years earlier; and by the differences in the age and lifestyle. Only 26.6 – 26.9% of the EpiPorto participants completed the secondary school or above, whereas the present study represents a younger and literate population.

In our study there was a low number of individuals consuming alcohol on a daily basis (4.1% vs 25.3 - 68.8%), but the number was considerably higher on a weekly basis (39.3% vs 14.4 - 11.8%). Such daily and weekly differences might again, be explained by the characteristics of the surveyed population. The academic community, particularly university students (which account for 64.4% of our surveyed individuals) have the social habit of drinking mainly over the weekends, whereas adults and seniors tend to drink on a daily basis. In fact, the consumption of alcoholic beverages (particularly wine and beer) reflects an important Portuguese cultural aspect, being widespread in the adult population (World Health Organization, 2014b). Our results, however, reveal that only 4.1% of the

individuals drink alcoholic beverages on a daily basis. According to the Portuguese National Council on Food and Nutrition, and based only on wine consumption, the recommended quantities for adult consumption are: maximum 2-3 units/day (28-42 g) for men and maximum of 1-2 units/day (14-28 g) for women (International Center for Alcohol Policies, 2015).

Coffee is also an important component of the Portuguese diet (INE, 2014); being consumed on a daily basis by 57.1% of the surveyed individuals and predominant over tea and infusions consumption (32%).

The daily consumption of food items with fast absorption sugars, such as refined sugar, cookies, pulp juices and soft drinks, demonstrates some changes in the dietary patterns in which this type of food items have assumed a greater presence. Such changes are particularly worrisome if we consider that when the consumption exceeds the adequate daily calorie intake (INE, 2014), they are associated with an increased risk of health problems including dental caries, obesity and diabetes (World Health Organization, 2015) that are arising in increasingly younger ages.

Therefore, the elevated frequency consumption of dairy products, fish and meat together with the intake of other products, such as deep-fried foods, sweets and canned food, suggests a slight deviation from the traditional Mediterranean diet. A similar conclusion was previously noticed by Chen and Marques-Vidal in 2007 (Chen and Marques-Vidal, 2007). These authors mentioned that the Portuguese diet was gradually moving away from the Mediterranean style.

Between men and women the percentages in the consumption frequencies (Table 2.1.2) were similar, for most of the listed food items. The main exceptions were observed for the daily consumption of sweets, pastry and tea with higher percentages for women; and pre-cooked meals, canned food and alcoholic beverages displaying higher weekly consumptions in men's diet. It is well established that this type of food items are associated with large number of health complications such as increased cardiovascular risk, metabolic syndrome obesity, hypertension, diabetes, dyslipidaemia and cancer (World Health Organization, 2003).

Particularly relevant for the consumption of canned foods is the intake of bisphenol A (BPA). This organic compound is an endocrine disruptor that is used as protective liner in metal cans to maintain the quality of canned foods and beverages and as a coating on

residential drinking water storage tanks (EFSA, 2015), however, the possibility of BPA migration from can is high (Sungur et al., 2014). Besides BPA other endocrine disrupting chemicals, including perfluorochemicals (PFCs), are of concern while consuming, for example precooked and fast food meals. PFCs are extensively used in food-packaging coatings and cooking materials (Domingo, 2012), therefore, the migration phenomenon is also considerable in this case.

These evidences, suggest that human exposure to such chemicals is higher when consuming these products, which further reinforces the importance of consuming fresh, local and seasonal products, typical of the Mediterranean diet.

2.1.5. Conclusions

This study provides an overview on the dietary habits of the academic community from the University of Aveiro. To the best of our knowledge, this is the first study on the dietary habits of an academic population in Portugal through the implementation of a food frequency questionnaire. Although this method does not allow to quantitatively evaluate the nutritive and feeding profile of the surveyed population it allows the analysis of the frequencies of consumption of different foods items. The obtained results demonstrate that this academic community does not strictly follow the recommendations for a healthy diet, nevertheless, the diet of the University of Aveiro surveyed volunteers still keeps many characteristics of the Mediterranean diet.

Acknowledgements

This work was supported by European Funds through COMPETE and by National Funds through the Portuguese Science Foundation (FCT) within projects PEst-C/MAR/LA0017/2013 and PEst-OE/SAU/UI0709/2014. Ana C. A. Sousa and Sónia D. Coelho acknowledge FCT for the grants SFRH/BPD/65884/2009 and SFRH/ BD/78168/2011 (supported by funding from the Human Potential Operational Programme POPH, inscribed in the National Strategic Reference Framework and partially subsidized by the European Social Fund). The authors also wish to deeply acknowledge all the volunteers' that participated in this study.

References

Cade, J.E., Burley, V.J., Warm, D.L., Thompson, R.L., Margetts, B.M., 2004. Food-frequency questionnaires: a review of their design, validation and utilisation. Nutr. Res. Rev. 17, 5-22.

Chen, Q., Marques-Vidal, P., 2007. Trends in food availability in Portugal in 1966–2003. Eur. J. Clin. Nutr. 46, 418-427.

Coelho, S.D., Sousa, A.C.A., Nogueira, A.J.A., Isobe, T., Tanabe, S., 2014. Levels of brominated flame retardants, polychlorinated biphenyls and organochlorine pesticides in diet samples from Aveiro University community, Portugal. Dioxin. Organohalogen Compounds, Madrid, Spain, pp. 740-743.

Domingo, J.L., 2012. Health risks of dietary exposure to perfluorinated compounds. Environ. Int. 40, 187-195.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 23.

INE, 2014. Balança Alimentar Portuguesa 2008-2012. Destaque - Informação à Comunicação, pp. 1-25.

International Center for Alcohol Policies, 2015. Table: International Drinking Guidelines. ICAP. http://icap.org/PolicyIssues/DrinkingGuidelines/StandardUnitsTable/tabid/253/Default.aspx. (accessed May, 2015).

Lopes, C., Oliveira, A., Santos, A.C., Ramos, E., Severo, M., 2006. Consumo Alimentar no Porto - Relatório Sumário. Faculdade de Medicina – Universidade do Porto, pp. 60.

Maruyama, K., Kokubo, Y., Yamanaka, T., Watanabe, M., Iso, H., Okamura, T., Miyamoto, Y., 2015. The reasonable reliability of a self-administered food frequency questionnaire for an urban, Japanese, middle-aged population: the Suita study. Nutr. Res. 35, 14-22.

OXFAM, 2014. Good Enough to Eat - Where in the world are the best and worst places to eat? Oxfam Media Briefing, pp. 6.

Petrillo, P.L., 2012. Biocultural diversity and the Mediterranean diet. in: Burlingame, B., Dernini, S., Nutrition and Consumer Protection Division, FAO (Eds.). Sustainable Diets and Biodiversity. FAO, pp. 222-293.

Poínhos, R., Oliveira, B.M.P.M., Correia, F., 2013. Eating behaviour patterns and BMI in Portuguese higher education students. Appetite 71, 314-320.

Sauvageot, N., Alkerwi, A.a., Albert, A., Guillaume, M., 2013. Use of food frequency questionnaire to assess relationships between dietary habits and cardiovascular risk factors in NESCAV study: validation with biomarkers. Nutr. J. 12, 143.

Sousa, A.C.A., Coelho, S.D., Pastorinho, M.R., Barros, A.R., Nogueira, A.J.A., Taborda-Barata, L., Tomohiko, I., Takahashi, S., Tanabe, S., 2014. Levels of organotin compounds in diet

samples from Portugal - preliminary results from a duplicate diet study. Dioxin. Organohalogen Compounds Madrid, Spain, pp. 637-640.

Sungur, Ş., Köroğlu, M., Özkan, A., 2014. Determinatıon of bisphenol a migrating from canned food and beverages in markets. Food Chem. 142, 87-91.

Vilone, G., Comiskey, D., Heraud, F., O'Mahony, C., 2014. Statistical method to assess usual dietary intakes in the European population. Food Addit. Contam. Part A 31, 1639–1651.

World Health Organization, 2003. Diet, nutrition and the prevention of chronic diseases. WHO Technical Report Series, pp. 148.

World Health Organization, 2006. Body Mass Index classification. Department of Nutrition for Health and Development. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (accessed April, 2015).

World Health Organization, 2014a. Food safety - Fact sheet N°399. http://www.who.int/mediacentre/factsheets/fs399/en/. (accessed February, 2015).

World Health Organization, 2014b. Global status report on alcohol and health - 2014. World Health Organization, Geneva, Switzerland, pp. 376.

World Health Organization, 2014c. Mean body mass index trends (age-standardized estimate) - Data by country. Global Health Observatory Data Repository - WHO. http://apps.who.int/gho/data/node.main.A904?lang=en. (accessed April, 2015).

World Health Organization, 2015. Guideline: Sugars intake for adult and children. World Health Organization, pp. 49.

2.2. Brominated flame retardants and organochlorine compounds in duplicate diet samples from a Portuguese academic community

Abstract

Concentrations of persistent organic pollutants (POPs), including polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDDs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), chlordane compounds (CHLs) and dichlorodiphenyltrichloroethane and its metabolites (DDTs), were measured in duplicate diet samples from 21 volunteers at a Portuguese academic community (University of Aveiro). Overall, the levels of the target compounds were low, with detection frequencies varying widely depending on the compounds and with brominated flame retardants (BFRs) registering the lowest detection frequencies. Among PCB congeners, nondioxin-like PCBs were predominant and detected in the majority of the samples. Organochlorine pesticides were also detected in the majority of the samples, with 100% detection for DDTs and HCHs.

Estimated daily intakes (EDIs) were calculated using lower and upper bound estimations, and in both cases values were far below the currently established tolerable daily intakes for PCBs and OCs and reference doses for PBDEs and HBCDDs.

Keywords: PBDEs, HBCDDs, PCBs, organochlorine pesticides, dietary intake, Portugal

2.2.1. Introduction

Exposure to environmental contaminants can occur through different pathways and, according to the European Food Safety Authority (EFSA, 2010, 2011b, a, 2012a), ingestion of food is particularly important for persistent organic pollutants (POPs). Human exposure and environmental contamination by POPs (e.g. organochlorine pesticides and brominated flame retardants (BFRs)), is a topic of significant importance worldwide. Because of their unique physicochemical properties, POPs are persistent in the environment and bioaccumulate through the food chain (Malarvannan et al., 2013; Mortimer, 2013). The list of POPs set by the Stockholm Convention includes polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDDs), polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDDs/PCDFs, not measured here), polychlorinated biphenyls (PCBs) and some organochlorine pesticides (OCs), such as hexachlorocyclohexanes (HCHs), hexachlorobenzene (HCB), chlordanes (CHLs) and dichlorodiphenyltrichloroethane (DDT) (Stockholm Convention, 2013). Besides the BFRs listed as POPs, there are other 'alternative' BFRs (emerging BFRs) that can be detected in food samples, for example, decabromodiphenyl ethane (DBDPE), 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE) and 1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane (TBECH) (EFSA, 2012b).

In order to test whether diet is a possible exposure route to the aforementioned compounds, we quantified the levels of these POPs and emerging BFRs in duplicate diet samples from a group of volunteers studying or working in the University of Aveiro, Portugal, and estimated the respective daily intakes.

2.2.2. Material and Methods

2.2.2.1. Sampling

Volunteers were selected during briefing sections held by the researchers at different departments of Aveiro University. A sampling kit was delivered to all the volunteers who agreed to participate (including students, researchers and teaching and nonteaching staff) by signing an informed consent. The participants (n = 21) completed a food frequency

questionnaire (FFQ) and were instructed to collect a small part representative of the portions of the dietary products consumed in all meals (including snacks) for 7 consecutive days. The samples were preserved daily in each participant's home freezer and, at the end of the week, delivered to the researchers who pooled together all samples from the same volunteer (7-day duplicate diet sample). The samples were then homogenised using a stainless steel hand blender and kept in amber glass vials at −20°C until freeze-drying and chemical analysis.

2.2.2.2. Chemical analysis

The PBDEs, HBCDDs, emerging BFRs, OCs and PCBs were analysed according to the method described by Asante et al. (2013).

After freeze-drying, an aliquot of approximately 20–30 g of sample was homogenised with anhydrous sodium sulphate (Nacalai Tesque Inc., Japan) and extracted using an SE-100 High Speed Solvent Extractor (Mitsubishi Chemicals, Japan) with a solution of acetone and hexane (1:1, *v/v*). The solvent was concentrated and filled up to 10 mL with hexane. From the obtained 10-mL extract, an aliquot (2 mL) was used for gravimetric lipid determination. The remaining extract was spiked with the internal cleanup spikes (IS: 5 ng each of $^{13}C_{12}$-labelled PBDEs, $^{13}C_{12}$-labelled PCBs and $^{13}C_{12}$-labelled OCs; 10 ng of $^{13}C_{12}$-labelled HBCDDs) and then subjected to a multi-layer silica gel column (150 ml of 25% dichloromethane in hexane, *v/v*). After this extra cleanup, the sample was subjected to gel permeation chromatography (GPC) for lipid removal. The GPC fraction containing the target compounds was concentrated and subjected to an activated silica gel column (Wakogel DX) for further cleanup and fractioning. The first fraction (eluted with 80 ml of 5% dichloromethane in hexane, *v/v*) contained PBDEs, PCBs and OCs, while the second fraction (100 ml of 25% dichloromethane in hexane, *v/v)* contained HBCDDs. Both fractions were concentrated individually and spiked with the respective syringe spikes to ensure the recoveries of surrogates ($^{13}C_{12}$-labelled BDE-126 and BDE-205 for PBDE IS, $^{13}C_{12}$-labelled BDE-139 for PCB IS, pentachloro anisole for OC IS and d_{18}-β-HBCDD for HBCDD IS). For QA/QC, with every batch of seven samples, a laboratory blank was analysed.

PBDEs, PCBs and emerging BFRs were Identified and quantified using a gas chromatograph (GC: Agilent 7980A) coupled with a mass spectrometer (MS: Agilent 5975C); OCs using a GC (Agilent 7890A) coupled with a triple quadrupole mass spectrometer (MS/MS: Agilent 7000) and HBCDDs using a liquid chromatograph (LC: Acquity Ultra Performance, Waters) coupled with a MS/MS (Quattro Micro API, Micromass). The limits of detection (LODs) were calculated as three times the standard deviation of background peaks in the procedural blanks. If a concentration of a given compound was below LOD, it was considered as zero for the total and mean concentrations calculations (lower bound (LB) estimations). In Table 2.2.1, the upper bound (UB) estimations are also depicted (the LOD value was attributed to the values <LOD).

2.2.2.3. Dietary Intake calculation

Estimated daily intakes (EDIs) of the target compounds were calculated considering that a person ingests daily 1867.2 g of food (data relative to the food ingestion rate in 2012, set by the National Institute of Statistics, Portugal (INE, 2014)), and the participants' body weights reported in the FFQ. LB and UB concentration values were also used to estimate the daily intakes (Table 2.2.1).

2.2.3. Results and Discussion

2.2.3.1. Concentrations in duplicate diet samples

BFR levels were low and below LODs in most of the samples (Table 2.2.1). Considering the PBDE congeners of primary interest (BDE-28, 47, 99, 100, 153, 154, 183 and 209) (EFSA, 2011b), BDE-209 showed higher detection frequency (66.7%, n = 14) and concentration (range: <LOD–0.22 ng g^{-1}, median: 0.031 ng g^{-1}), followed by BDE-47 and BDE-99 that were detected in 4 (19.0%) and 2 (9.5%) samples with values ranging from <LOD to 0.014 ng g^{-1} and <LOD and 0.010 ng g^{-1}, respectively; the median concentrations of BDE-47 and BDE-99 were below LOD. The effect of BDE-209 on the total concentration of PBDEs can be easily noted when comparing the concentrations of

the sum of the eight primary congeners (mean: 0.046 ng g^{-1}) with the concentration resulting from the sum of the tri-hepta-BDEs (mean: 0.0024 ng g^{-1}) (Table 2.2.1). In fact, the sum of tri-hepta-BDEs is one order of magnitude lower than the sum of the eight primary congeners.

Table 2.2.1. Lower bound (LB) and upper bound (UP) concentrations (ng g^{-1} ww) of the target organohalogen compounds in the 21 duplicate diet samples and participants' estimated dietary daily intakes (ng day^{-1} and ng kg-bw^{-1} day^{-1}).

		Concentration (ng g^{-1} ww)	Daily intake[k] (ng day^{-1})	Daily intake[l] (ng kg-bw^{-1} day^{-1})
PBDEs[a]				
LB	Range	<LOD–0.23	0–440	0– 9.1
	Mean (Median)	0.049 (0.036)	91 (67)	1.6 (1.1)
UB	Range	0.30–0.63	560–1200	6.8–24
	Mean (Median)	0.38 (0.35)	710 (650)	12 (11)
BDE_8[b]				
LB	Range	<LOD–0.22	0–420	0–8.7
	Mean (Median)	0.046 (0.035)	85 (65)	1.5 (0.98)
UB	Range	0.059–0.28	110–520	1.5–11
	Mean (Median)	0.099 (0.090)	190–170	3.1 (2.7)
Tri-hepta-BDE[c]				
LB	Range	<LOD–0.021	0–40	0–0.79
	Mean (Median)	0.0024 (<LOD)	4.6 (0)	0.082 (0)
UB	Range	0.037–0.060	68–110	0.80–2.2
	Mean (Median)	0.046 (0.042)	86 (78)	1.4 (1.4)
HBCDDs[d]				
LB	Range	<LOD–1.2	0–2200	0–37
	Mean (Median)	0.062 (<LOD)	120 (0)	2.0 (0)
UB	Range	0.017–1.2	32–2200	0.37–37
	Mean (Median)	0.079 (0.021)	150 (40.0)	2.5 (0.70)
PCBs				
LB	Range	<LOD–0.95	0–1800	0–36
	Mean (Median)	0.26 (0.17)	490 (320)	8.0 (5.5)
UB	Range	0.071–1.4	130–2600	2.4–53
	Mean (Median)	0.48 (0.42)	900.0 (790)	15 (11)
PCB_{18}[e]				
LB	Range	<LOD–0.60	0–1100	0–22
	Mean (Median)	0.12 (0.091)	230 (170)	3.8 (3.0)
UB	Range	0.020–0.73	37–1400	0.67–27
	Mean (Median)	0.18 (0.17)	330 (320)	5.6 (4.7)
ndl-PCBs[f]				
LB	Range	<LOD–0.54	0–1000	0–20.0
	Mean (Median)	0.11 (0.076)	200 (140)	3.3 (2.5)

		Concentration (ng g^{-1} ww)	Daily intake[k] (ng day^{-1})	Daily intake[l] (ng kg-bw^{-1} day^{-1})
UB	Range	0.012–0.57	23–1100	0.41–21
	Mean (Median)	0.12 (0.087)	220 (160)	3.7 (3.1)
dl-PCBs[g]				
LB	Range	<LOD–0.052	0–97	0–1.9
	Mean (Median)	0.014 (0.0097)	26 (18)	0.45 (0.27)
UB	Range	0.0080–0.16	14–300.0	0.23–6.0
	Mean (Median)	0.058 (0.027)	110 (49)	1.9 (0.96)
HCHs[h]				
LB	Range	0.0093–0.16	17–290	0.26–4.6
	Mean (Median)	0.043 (0.029)	80.0 (54)	1.4 (0.78)
UB	Range	0.032–0.17	60–310	0.90–4.9
	Mean (Median)	0.059 (0.041)	110 (76)	1.9 (1.3)
HCB				
LB	Range	<LOD–0.062	0–120	0–2.2
	Mean (Median)	0.024 (0.024)	45 (45)	0.77 (0.62)
UB	Range	0.0060–0.062	11–120	0.16–2.2
	Mean (Median)	0.025 (0.024)	47 (45)	0.79 (0.62)
CHLs[i]				
LB	Range	<LOD–1.0	0–2000	0–30.0
	Mean (Median)	0.12 (0.028)	220 (53)	3.5 (1.0)
UB	Range	0.028–1.0	53–2000.0	0.62–30.0
	Mean (Median)	0.14 (0.046)	260 (86)	4.1 (1.7)
DDTs[j]				
LB	Range	0.11–0.73	200–1400	2.4–21
	Mean (Median)	0.35 (0.38)	660 (710)	11 (12)
UB	Range	0.12–0.74	230–1400	2.7–22
	Mean (Median)	0.37 (0.40)	690 (740)	11 (12)

[a] Sum of all congeners
[b] Sum of BDE-28, 47, 99, 100, 153, 154, 183, 209
[c] Sum of BDE-28, 47, 99, 100, 153, 154, 183
[d] Sum of α, β, γ-HBCDD
[e] Sum of dioxin-like PCBs and nondioxin-like PCBs
[f] Sum of nondioxin-like PCBs (28, 52, 101, 138, 153 and 180)
[g] Dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189)
[h] Sum of α, β, γ , δ-HCH
[i] Sum of trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor and oxychlordane
[j] Sum of o,p-DDT, o,p-DDD, o,p-DDE, p,p'-DDT, p,p'-DDD, p,p'-DDE
[k] Estimated considering the detected concentrations and 1867.2 g/inhab/day as the daily edible per capita in 2012 (INE, 2014)
[l] Estimated considering the detected concentrations, 1867.2 g/inhab/day as the daily edible per capita and each participant body weight.

To the best of the authors' knowledge, only five studies have addressed the levels of PBDEs in duplicate diet samples (Harrad et al., 2004; Fromme et al., 2009; Roosens et al., 2009b; De Filippis et al., 2014; Fujii et al., 2014). Roosens et al. (2009b) analysed PBDEs

in duplicate diet samples of 19 students from the University of Antwerp, Belgium, and the BDE-209 and tri-hepta-BDEs levels were slightly higher than ours and BDE-47 and 99 were also the major congeners contributing to the sum of tri-hepta-BDEs. As seen in Table 2.2.2, it is impossible to compare the total PBDE levels among studies, as different congeners were used to quantify the total BDEs. However, excluding the BDE-209 (not available for all studies), BDE-47 and BDE-99 were the major contributing congeners to the total concentrations in all studies. These observations suggest that Portuguese diet is less contaminated by PBDEs than other European countries.

Table 2.2.2. Comparison of PBDE concentrations in duplicate diet samples from Portugal with other European countries.

References	Country	n	BDE-47	BDE-99	BDE-209	ΣBDEs	Units
Harrad et al. (2004)	UK*	10	66.8	63.8	n.a.	181[a]	pg g^{-1} dw
Fromme et al. (2009)	Germany**	350	47–5193	34–5062	n.a.	n.a.	pg g^{-1} lw
Roosens et al. (2009b)	Belgium*	19	n.a.[d]	n.a.[d]	0.139	0.01[b]	ng g^{-1} ww
De Filippis et al. (2014)	Italy***	1	0.00385	0.00243	<0.0743	0.0937[c]	ng g^{-1} ww
This study	Portugal*	21	<LOD	<LOD	0.031	0.035[c]	ng g^{-1} ww

*median concentrations; ** range concentrations; ***one pooled sample composed of eight duplicate diet samples.
[a] sum of BDEs 47, 99, 100, 153 and 154; [b] sum of BDEs 28,47, 99, 100, 153, 154 and 183; [c] sum of BDEs 28,47, 99, 100, 153, 154, 183 and 209; [d] although the authors do not describe the concentrations of BDE 47 and 99, they consider them as major contributing congeners.
n.a. information not available; dw: dry weight, lw: lipid weight, ww: wet weight.

For HBCDDs, the levels of the individual isomers (α, β, γ-HBCDD) were mostly below LOD, and α-HBCDD was the most frequent isomer (23.8%, *n* = 5). The total concentrations of HBCDDs varied from <LOD to 1.2 ng g^{-1} ww (median: <LOD, Table 2.2.1). Similar results were obtained in the duplicate diet samples of 16 Belgian students from the University of Antwerp (range: <0.01–0.35 ng g^{-1} ww) (Roosens et al., 2009a), which is the only reported duplicate diet study for HBCDDs in Europe.

The concentrations of emerging BFRs (DBDPE, BTBPE and TBECH) were all below LOD and therefore the results will not be further discussed.

The total concentrations of all PCB congeners as well as the concentrations of dioxin-like PCBs (dl-PCBs: 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) and

nondioxin-like PCBs (ndl-PCBs: 28, 52, 101, 138, 153 and 180) are shown in Table 2.2.1. The most prevalent PCB congeners were nondioxin-like PCBs, 138 (95.2%, *n* = 20), 153 (95.2%, *n* = 20), 180 (81.0%, *n* = 17) and 101 (76.2%, *n* = 16). When comparing with dioxin-like PCBs (median: 0.0097 ng g^{-1} ww), these nondioxin-like PCBs (median: 0.076 ng g^{-1} ww) constitute the larger proportion of the total PCBs (Figure 2.2.1).

There are several market-basket surveys describing the PCB levels in food; however, in Europe, only two studies are available for duplicate diet (e.g. Roosens et al., 2010; De Filippis et al., 2014). Roosens et al. (2010) revealed the levels of PCBs in 19 duplicate diet samples from Antwerp University students and showed that PCB 138 and 153 were the major contributors to the total PCB concentrations, similar to our survey. These two ndl-PCB congeners were also the most prevalent in samples of food items purchased from Portuguese retail shops, including salmon, butter and cabbage (Zuccato et al., 2008). In the survey conducted by De Filippis et al. (2014), the results have been reported for one pooled sample in terms of the sum of dl-PCBs, PCDDs and PCDFs, and hence it was impossible to compare with our results.

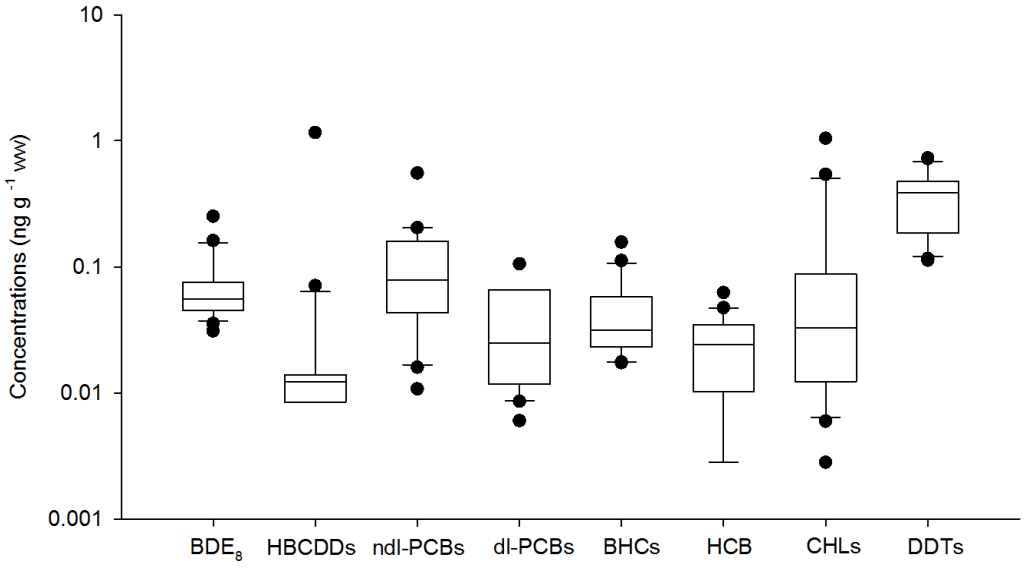

Figure 2.2.1. Boxplot summarising the variation in concentrations of the target compounds in 21 duplicate diet samples. Outliers, maximum, minimum, median and the 25th and 75th percentiles are presented (ng g^{-1} ww). For those samples in which some compounds were below the LOD, we considered those values (only for the graphical approach) as half the LOD.

Concerning the high persistence of OCs, although their agricultural use has been banned, these contaminants were still detected in the majority of the duplicate diet samples, with DDTs and HCHs being detected in 100% of the samples, and HCB and CHLs in 85.7% (*n* = 18) and 71.4% (*n* = 15), respectively. The higher detection frequency, mean (0.35 ng g^{-1}) and median (0.38 ng g^{-1}) values of DDTs comparing with the other OCs have revealed their leading presence in the analysed diet samples (Figure 2.2.1), regardless of the ban on their use as a pesticide in the European Union since 1986 (EFSA, 2006).

As mentioned earlier, there is limited information on the levels of POPs in duplicate diet samples. Such scarcity of data is probably related with the difficulties in collecting this type of samples. Nevertheless, there are several surveys describing the levels of POPs in selected food items, namely fish, meat and dairy products (Gasull et al., 2011). The available data suggest that distribution of BFRs, PCBs and OCs varies among foodstuff, wherein fish, meat and dairy products have been reported in several surveys as important contributors to total dietary intake of POPs (Gasull et al., 2011). Therefore, the low frequencies of detection (e.g. BFRs) and low concentrations observed in this study are possibly associated with intrinsic characteristics of the collection procedure. By homogenising proportional parts of complete meals, the detection of POPs in this type of samples can be compromised as the quantities of less-contaminated ingredients (e.g. carbohydrates) exceeded the high-contaminated ones (e.g. fish, meat and dairies). Nevertheless, duplicate diet surveys ensure that the measured levels reflect all the different contamination sources (Vestergren and Cousins, 2013) and therefore allow to quantify the actual levels to which participants are exposed (that can be from the food item itself, or introduced in the food by packing material and cooking processes).

2.2.3.2. Dietary daily intakes

In order to understand human exposure to these POPs through food ingestion, the dietary daily intakes were estimated from the concentrations detected in the duplicate diet samples (using both LB and UB estimations) and 1867.2 g as the daily edible per capita reported in 2012 (INE, 2014) (Table 2.2.1). The EDIs for PBDEs and LB and UB estimations varied from 0 to 440 and 560 to 1200 ng day^{-1}, respectively. For HBCDDs, they varied from 0 to 2200 and 32 to 2200 ng day^{-1} for LB and UB estimations, respectively. Although the

median EDI for PBDEs (67–650 ng day^{-1}, LB–UB) was higher than the median value of HBCDDs (0–40 ng day^{-1}, LB and UB), the highest EDI (2200 ng day^{-1}) was found for HBCDDs in one sample. This can be explained by the fact that the use of HBCDDs in the European Union continued until August 2015, while the use of PBDEs was phased out in 2004 (Penta and Octa formulations) and 2008 (Deca-BDE) (Coelho et al., 2014). Considering the LB estimations, the mean and median EDIs for PBDEs in our survey were lower than the values reported in the Belgian (Roosens et al., 2009b) and German (Fromme et al., 2009) duplicate diet studies, but in the case of HBCDDs, the highest EDI observed in this study was two orders of magnitude higher than the value described by Roosens et al. (2009a) in Belgium (range: 1.2–20 ng day^{-1}, median: 5.5 ng day^{-1}). Because of the uncertainties and deficiencies in the PBDE and HBCDD toxicological databases, their tolerable daily intakes (TDIs) are not established (Lambropoulou et al., 2010). However, the EDIs per kilogram of body weight, which was obtained from each participant, observed in this study were well below the reference doses for BDE-47 (100 ng kg-bw^{-1} day^{-1}), BDE-99 (100 ng kg-bw^{-1} day^{-1}) and BDE-209 (7000 ng.kg-bw^{-1} day^{-1}) set by the US EPA (United States Environmental Protection Agency), and for HBCDDs (200 ng kg-bw^{-1} day^{-1}) set by the US National Research Council (Björklund, 2011).

EDIs of PCBs varied from 0 to 1800 and 130 to 2600 ng day^{-1} for LB and UB estimations, respectively (median: 320–790 ng day^{-1}, LB–UB) (Table 2.2.1) and were higher than the values reported by Roosens et al. (2010) with the sum of PCB 118, 138, 153, 180 and 170 ranging from 40 to 204 ng day^{-1} (median: 133 ng day^{-1}). The EDIs per kilogram of body weight of PCBs were lower than the TDI of 1 µg kg-bw^{-1} day^{-1} set by the United Nations Food and Agriculture Organization and World Health Organization (FAO/WHO) (Simmonds et al., 2002). For the DL-PCBs, we further calculated the WHO TEQ values (van den Berg et al., 2006). The average EDIs for the sum of dl-PCBs were 0.41 and 110 pg WHO TEQ kg-bw^{-1} $week^{-1}$ for LB and UB estimates, respectively. Whilst the LB values are much lower than the tolerable weekly intake (14 pg WHO TEQ kg-bw^{-1} $week^{-1}$) set by the EU Scientific Committee on food and the provisional tolerable monthly intake established by the Joint FAO/WHO Expert Committee on Food Additives (70 pg WHO TEQ kg-bw^{-1} $month^{-1}$) (Malisch and Kotz, 2014), the UB estimates are higher. Nevertheless because of huge differences between LB and UB estimations (over three

orders of magnitude), a high degree of uncertainty exists and these results should be carefully addressed.

For OCs, the EDIs per kilogram of body weight (Table 2.2.1) were lower than the TDIs established by WHO: 8, 0.17, 0.5 and 20 μg kg-bw^{-1} day^{-1} for HCH, HCB, CHLs and DDTs, respectively (Simmonds et al., 2002; Falco et al., 2004; EFSA, 2007). These observations indicate that health risk by POPs via diet is relatively low for the Portuguese population.

2.2.4. Conclusions

Overall, the duplicate diet samples disclosed low concentrations of BFRs, PCBs and OCs. In fact, the levels of PBDEs and HBCDDs in several samples were below the LOD, which indicates that diet is not an important source of these BFRs in the studied population. Nevertheless, PCBs and organochlorine pesticides were detected in most of the samples, with DDTs being found in all the samples analysed.

Our results also showed that the EDIs of the selected POPs (PCDDs/PCDFs are not measured here) were far below the reference doses established for PBDEs and HBCDDs and below the TDIs set for PCBs and OCs.

Acknowledgements

The authors acknowledge the volunteers who participated in this study. The assistance of R. Okubo and S. Nishioka during chemical analysis is deeply acknowledged as well as the assistance provided by Dr N. M. Tue during data analysis. The authors also thank Dr Subramanian, Emeritus Professor, Bharathidasan University, India, for critically going through the manuscript. This study was supported by European Funds through COMPETE and National Funds through the Portuguese Science Foundation (FCT) within projects PEst-C/MAR/LA0017/2013 and PEst-OE/SAU/UI0709/2014. Ana C. A. Sousa and Sónia D. Coelho acknowledge FCT for the grants SFRH/BPD/65884/2009 and SFRH/ BD/78168/2011 (supported by funding from the Human Potential Operational Programme POPH, inscribed in the National Strategic Reference Framework and partially subsidised by the European Social Fund).

References

Asante, K.A., Takahashi, S., Itai, T., Isobe, T., Devanathan, G., Muto, M., Agyakwah, S.K., Adu-Kumi, S., Subramanian, A., Tanabe, S., 2013. Occurrence of halogenated contaminants in inland and coastal fish from Ghana: Levels, dietary exposure assessment and human health implications. Ecotox. Environ. Safe. 94, 123-130.

Björklund, J., 2011. Brominated flame retardants and perfluoroalkyl acids in Swedish indoor microenvironments: Implications for human exposure. Applied Environmental Science. Stockholm University, pp. i - x, 1 - 47.

Coelho, S.D., Sousa, A.C.A., Isobe, T., Tanabe, S., Nogueira, A.J.A., 2014. Flame retardants in indoor dust - A review on the levels of polybrominated diphenyl ethers and hexabromocyclododecanes. Curr. Org. Chem. 18, 2218-2230.

De Filippis, S.P., Brambilla, G., Dellatte, E., Corrado, F., Esposito, M., 2014. Exposure to polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs) and polybrominated diphenyl ethers (PBDEs) through the consumption of prepared meals in Italy. Food Addit. Contam. Part A. 31, 1114-1126.

EFSA, 2006. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to DDT as an undesirable substance in animal feed. EFSA J. 1 - 69.

EFSA, 2007. Chlordane as undesirable substance in animal feed (1) Scientific Panel on Contaminants in the Food Chain. EFSA J. 1-53.

EFSA, 2010. Results of the monitoring of non dioxin-like PCBs in food and feed. EFSA J. 35.

EFSA, 2011a. Scientific Opinion on Hexabromocyclododecanes (HBCDDs) in Food. EFSA J. 118.

EFSA, 2011b. Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA J. 274.

EFSA, 2012a. Scientific Opinion on Brominated Flame Retardants (BFRs) in Food: Brominated Phenols and their Derivatives. EFSA J. 42.

EFSA, 2012b. Scientific Opinion on Emerging and Novel Brominated Flame Retardants (BFRs) in Food. EFSA J. 133.

Falco, G., Bocio, A., Llobet, J.M., Domingo, J.L., Casas, C., Teixido, A., 2004. Dietary intake of hexachlorobenzene in Catalonia, Spain. Sci. Total. Environ. 322, 63-70.

Fromme, H., Körner, W., Shahin, N., Wanner, A., Albrecht, M., Boehmer, S., Parlar, H., Mayer, R., Liebl, B., Bolte, G., 2009. Human exposure to polybrominated diphenyl ethers (PBDE), as evidenced by data from a duplicate diet study, indoor air, house dust, and biomonitoring in Germany. Environ. Int. 35, 1125-1135.

Fujii, Y., Nishimura, E., Kato, Y., Harada, K.H., Koizumi, A., Haraguchi, K., 2014. Dietary exposure to phenolic and methoxylated organohalogen contaminants in relation to their concentrations in breast milk and serum in Japan. Environ. Int. 63, 19-25.

Gasull, M., Bosch de Basea, M., Puigdomènech, E., Pumarega, J., Porta, M., 2011. Empirical analyses of the influence of diet on human concentrations of persistent organic pollutants: A systematic review of all studies conducted in Spain. Environ. Int. 37, 1226-1235.

Harrad, S., Wijesekera, R., Hunter, S., Halliwell, C., Baker, R., 2004. Preliminary Assessment of U.K. Human Dietary and Inhalation Exposure to Polybrominated Diphenyl Ethers. Environ. Sci. Technol. 38, 2345-2350.

INE, 2014. Balança Alimentar Portuguesa 2008-2012. Destaque - Informação à Comunicação, Instituto Nacional de Estatística, pp. 1-25.

Lambropoulou, D., Evgenidou, E., Christophoridis, C., Bizani, E., Fytianos, K., 2010. Flame Retardants. in: Nollet, L.M.L. (Ed.). Analysis of Endocrine Disrupting Compounds in Food. Wiley-Blackwell, pp. 377-411.

Malarvannan, G., Dirinck, E., Dirtu, A.C., Pereira-Fernandes, A., Neels, H., Jorens, P.G., Gaal, L.V., Blust, R., Covaci, A., 2013. Distribution of persistent organic pollutants in two different fat compartments from obese individuals. Environ. Int. 55, 33-42.

Malisch, R. and Kotz, A., 2014. Dioxins and PCBs in feed and food — Review from European perspective. Sci. Total. Environ. 491-492, 2-10.

Mortimer, D.N., 2013. 1 - Persistent organic pollutants in foods: science, policy and regulation. in: Rose, M., Fernandes, A. (Eds.). Persistent Organic Pollutants and Toxic Metals in Foods. Woodhead Publishing, pp. 3-19.

Roosens, L., Abdallah, M.A.-E., Harrad, S., Neels, H., Covaci, A., 2010. Current Exposure to Persistent Polychlorinated Biphenyls (PCBs) and Dichlorodiphenyldichloroethylene (p,p'-DDE) of Belgian Students from Food and Dust. Environ. Sci. Technol. 44, 2870-2875.

Roosens, L., Abdallah, M.A.E., Harrad, S., Neels, H., Covaci, A., 2009a. Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: Preliminary results. Environ. Health Persp. 117, 1707-1712.

Roosens, L., Abdallah, M.A.E., Harrad, S., Neels, H., Covaci, A., 2009b. Factors influencing concentrations of polybrominated diphenyl ethers (PBDEs) in students from Antwerp, Belgium. Environ. Sci. Technol. 43, 3535-3541.

Simmonds, M.P., Haraguchi, K., Endo, T., Cipriano, F., Palumbi, S.R., Troisi, G.M., 2002. Human health significance of organochlorine and mercury contaminants in Japanese whale meat. J. Toxicol. Environ. Health A. 65, 1211-1235.

Stockholm Convention, 2013. Listing of POPs in the Stockholm Convention. http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx. (accessed August, 2015).

van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. Toxicol. Sci. 93, 223-241.

Vestergren, R., Cousins, I.T., 2013. 12 - Human dietary exposure to per- and poly-fluoroalkyl substances (PFASs). in: Rose, M., Fernandes, A. (Eds.). Persistent Organic Pollutants and Toxic Metals in Foods. Woodhead Publishing, pp. 279-307.

Zuccato, E., Grassi, P., Davoli, E., Valdicelli, L., Wood, D., Reitano, G., Fanelli, R., 2008. PCB concentrations in some foods from four European countries. Food Chem. Toxicol. 46, 1062-1067.

2.3. Lead in duplicate diet samples from an academic community

Abstract

Lead is a naturally occurring element that with the advent of the industrial era became a serious environmental and public health issue. Leaded gasoline, lead based paints, use of lead in plumbing and water pipes, ceramics with lead-containing glazes and tobacco smoke are potential sources of lead exposure for humans. Despite these multiple sources, food is still considered the most important one for the general non-smoking population.

Hence, in the present study, the dietary intake of lead was determined in duplicate diet samples provided by 30 participants working or studying at University of Aveiro, Portugal. Pb was detected in all the analysed samples with values ranging between 0.009 and 0.10 mg kg^{-1}ww which correspond to estimated daily intakes between 0.22 and 3.5 μg kg-bw^{-1} day^{-1}. Risk estimations disclose that at least 3.3% and 26.7% of the participants might suffer cardiovascular and nephrotoxic effects, respectively.

Keywords: Food; Duplicate diet samples; Estimated daily intakes; Cardiovascular effects; Nephrotoxicity.

2.3.1. Introduction

Metals are ubiquitous and naturally present in the environment, however anthropogenic activities are responsible for increased emissions of these contaminants into the ecosystems (Nordberg et al., 2007). Lead (Pb) is a non-essential metal for which the major historical sources were lead based paints, gasoline, piping used in tap water distribution and several consumer products (EFSA, 2012). Over the last few decades, lead emissions in developed countries have decreased significantly due to the prohibition of leaded gasoline and lead based paints (Pavón et al., 2015). Despite such restrictions, Pb poisoning is still a matter of great concern. This metal is a well-known neurotoxicant, particularly for children (Chiodo et al., 2007; ATSDR, 2007). It is also associated with hypertension and chronic kidney disease (EFSA, 2010). According to the International Agency for Research on Cancer (IARC) inorganic Pb compounds are classified as "probably carcinogenic" to humans (Group 2 A) (IARC, 2006). Hence, the establishment of tolerable intake levels is of upmost importance. In Europe, the European Food Safety Agency (EFSA) is responsible to set the tolerable doses for environmental contaminants. Lead toxicity was revised in 2010 by the EFSA Panel on Contaminants in the Food Chain - CONTAM Panel (EFSA, 2010). The CONTAM Panel considered that the Provisional Tolerable Weekly Intake (PTWI) set by JECFA (Joint FAO/WHO Expert Committee on Food Additives) for Pb (25 μg kg-bw^{-1} week^{-1}) was no longer appropriate, as there was no evidence for a threshold for critical effects induced by this metal (Chiodo et al., 2007; EFSA, 2010). Therefore, no safe lead level could be established (EFSA, 2010). However the Panel identified three endpoints as the critical effects for risk assessment: developmental neurotoxicity in young children (DN), systolic blood pressure (SBP) and chronic kidney disease (CKD) in adults. They determined the 95th percentile lower confidence limit of the benchmark dose of 1% extra risk ($BMDL_{01}$) for DN of 12 μg L^{-1} of Pb in blood which corresponds to an intake of 0.50 μg kg-bw^{-1} day^{-1} ($BMDL_{01}$, 12 (0.50)); for SBP the benchmark dose was set at 36 μg L^{-1} of Pb in blood corresponding to an intake of 1.50 μg kg-bw^{-1} day^{-1} ($BMDL_{01}$, 36 (1.50)); and for CKD the $BMDL_{10}$ (of 10% extra risk) was set at 15 μg L^{-1} of Pb in blood which corresponds to dietary intake values of 0.63 μg kg-bw^{-1} day^{-1} ($BMDL_{10}$, 15 (0.63) (EFSA, 2010). These blood Pb levels were calculated using the Carlisle and Wade (1992) equation, and USEPA's IEUBK model (US-EPA, 2010), respectively. The Carlisle and Wade (1992)

equation estimates blood Pb in adults, and considers five pathways – (1) dietary, (2) drinking water, (3) soil and dust ingestion, (4) inhalation, and (5) dermal contact. Each of these pathways is represented by its own equation that relates an incremental increase in blood Pb level to a concentration in a medium, using contact rates and pathway specific constants (Carlisle and Wade, 1992). The median blood Pb concentration is a result of the contributions of all the exposure pathways (Carlisle and Wade, 1992). However, the CONTAM panel (EFSA, 2010) considered the drinking water and the dermal contact pathways as non-relevant, and therefore they calculated blood lead levels [Pb_B] using an abridged form of the original equation:

Equation 1:

$$[Pb_B] = [Pb_{diet}] \cdot contact\ rate \cdot 0.4 + [Pb_{soil+dust}] \cdot 0.025 \cdot 0.18 + [Pb_{air}] \cdot 16.4$$

where, [Pb_B]: Blood Pb level (μg L^{-1}); [Pb_{diet}]: Pb level in diet (μg kg^{-1} diet); Contact rate: amount of diet (kg diet day^{-1});[$Pb_{soil\ +\ dust}$]: Pb level in soil and dust (mg kg^{-1}); [Pb_{air}]: air Pb level (μg m^{-3}).

The coefficients used in Eq. (1), namely the dietary constant (0.4 μg Pb L^{-1} blood/μg Pb day^{-1}), the soil + dust constant (0.18 μg Pb L^{-1} blood/μg ingested Pb day^{-1}) and the inhalation constant (16.4 μg Pb L^{-1}/μg m^{-3}) were the ones included by Carlisle and Wade (1992) in the original equation whereas the constant 0.025 was included by EFSA (see explanation below). These constants were obtained as follows: (i) the dietary constant (0.4 μg Pb L^{-1} blood/μg Pb day^{-1}) was an empirically derived value based in studies of plant uptake from Pb contaminated soils, as recommended by US-EPA (US-EPA, 1986). (ii) The soil + dust constant (0.18 μg Pb L^{-1} blood/μg ingested Pb day^{-1}) represents 44% of the lead ingested with food or water, since this was the highest bioavailability value registered by Chaney et al. (1989) for soil lead concentrations inferior to 1000 mg kg^{-1}. (iii) The constant 0.025 was not included in the equation originally presented by Carlisle and Wade (1992) as they did not integrate dust ingestion in their original calculations. It is our assumption that the constant 0.025 was included by EFSA experts in the calculations in order to account for the percentage of bioavailable lead reaching the blood via ingested dust (the CONTAM Panel document offers no explanation). (iv) The inhalation constant (16.4 μg Pb L^{-1}/μg m^{-3}) refers to continuously breathed air (adults) that was based on

results of experimental exposures and epidemiological studies. These studies considered other pathways rather than inhalation to calculate lead airborne concentrations. The slopes found by those studies varied from 1.25 to 2.14 in adults at Pb atmospheric concentrations up to 5 μgm^{-3} (US-EPA, 1986).

The EFSA CONTAM Panel when estimating the blood Pb levels for the endpoints SBP, CKD, and ND considered the exposure from air and from soil and dust negligible and therefore the numerical values indicated for these endpoints (36 $\mu g\ L^{-1}$ for SBP, 15 $\mu g\ L^{-1}$ for CKD and 12 $\mu g\ L^{-1}$ for ND) did not include these air and soil/dust terms and the estimation of blood Pb levels was solely based on the dietary intake.

Considering that food ingestion is the main exposure pathway to Pb in the general non-smoking population (EFSA, 2012) the aim of this study is to characterize the exposure of a Portuguese academic community (University of Aveiro, Portugal) to this metal through food consumption by evaluating its levels in duplicate diet samples.

2.3.2. Material and Methods

Between May and June 2012 a convenience sampling campaign was launched at University of Aveiro, Portugal. Several briefing sessions were conducted in selected departments in order to disseminate information about the project and to recruit volunteers. All the individuals that volunteered (including students, researchers, teaching and nonteaching staff) received a sampling kit and signed an informed consent to confirm their willingness to participate. Details on the sampling procedure are provided in a previous publication (Coelho et al., 2016) and are largely based on the procedure described by Roosens et al. (2009). In brief, each participant collected a small representative portion of the dietary products consumed in all meals (including snacks) during a week (7 consecutive days), while maintaining their regular dietary habits and completed a questionnaire. Samples were preserved daily in the participants' domestic freezers and delivered to the research team at the end of the week. At the laboratory, the samples from each participant (composed of all the samples collected daily by each volunteer during the 7 days) were pooled together, homogenised through a stainless-steel hand blender and kept in centrifuge tubes in the dark at −20 °C. Before chemical analysis, the samples were freeze dried and the moisture content determined.

An aliquot of about 200 mg of each duplicate diet sample (n=30) was placed in a cleaned Teflon screw top digestion vessel with 5 mL of concentrated nitric acid (Wako Pure Chemical Industries, Ltd.). Samples were digested in a microwave system (Ethos D, Milestone). Once cooled, the digested samples were diluted to 50 mL with Milli-Q water. Measurements were performed using an inductively coupled plasma mass spectrometer (ICP-MS; Agilent 7500cx, Agilent Technologies). For quality assurance and quality control certified calibration standards (purchased from Wako Pure Chemical Industries, Japan) were used, and analysis of matrix spikes performed. Rhodium was used as internal standard. Reagent blanks (nitric acid) and the certified standard reference material Oyster Tissue (NIST SRM1566b; Pb Certified Mass Fraction Value = 0.308 ± 0.009 mg kg^{-1}) were analysed with each batch of samples and the recoveries varied between 110 and 115%. Detection limit of the technique (for ^{208}Pb and determined based on three times the standard deviation of ten successive measurements of the blank) was 0.05 μg L^{-1}.

Estimated daily intakes (EDIs) were calculated considering 1.867 kg $inhab^{-1}$ day^{-1} as the daily edible consumption per capita in Portugal set by the National Institute of Statistics, Portugal (INE, 2014) and the participants' body weights reported in the questionnaire (45–95 kg). The influence of sex, occupation and age were evaluated by means of Mann-Whitney *U* test, Kruskal-Wallis test and Spearman Correlation, respectively. The analysis were performed using IBM SPSS Statistics 20 for a significance level of 0.05.

The blood lead concentrations were estimated using two different approaches: one considering all exposure sources as depicted in Eq. (1) and the other one excluding exposure from air and from soil and dust, as performed by EFSA (see Introduction section). The parameters used in the calculations were: [Pb_{diet}]=concentrations of lead detected in the samples (mg kg^{-1}); Contact rate: 1.867 kg $inhab^{-1}$ day^{-1} (INE, 2014); [Pb_{air}]: 1 μg Pb m^{-3}, value for the Aveiro region calculated from Freitas et al. (2005); Soil: 71.70 mg kg^{-1}, a geometric mean calculated from data by Patinha et al. (2012); House Dust: 79.77 mg kg^{-1}, value obtained from a default mass fraction outdoor-indoor dust conversion factor of 0.70 (US-EPA, 2008). This estimated value is in good agreement with previous studies performed by the team where the geometric mean of 84.0 mg kg^{-1} was obtained for a house dust survey in Aveiro households (Sousa et al., 2012).

Considering that children living in the same household as the participants are highly likely to follow the same diet as the adults, we further estimated children blood Pb levels using

the Integrated Exposure Uptake Biokinetic Model (IEUBK) for Pb in children. The IEUBK model used (IEUBKwin v1.1 build 11, Windows® version) includes, besides diet, indoor air, drinking water, soil and house dust. For this work, the topmost age range available in the IEUBK estimation model (6–7 years old) was used. Parameters used in the model were: Diet: intake calculated on the basis of 1/3 of the contact rate of adults (DGS, 2014) = 0.6224 kg child^{-1} day^{-1}; Air: 30% of the outdoor value (US-EPA, 2007) set at 1 μg Pb m^{-3} (Freitas et al., 2005); Drinking Water: 2.0 μg Pb L^{-1}, a conservative value extrapolated from reports by ADRA (the municipal water distribution system for the Aveiro Region) that report levels <3 μg Pb L^{-1} (ADRA, 2014); Soil and House Dust: similar to those stated above. Since local variations are not likely, several default values of the model for the 6–7 years old range regarding Air [time outdoors (4 h), ventilation rate (7 m^3 day^{-1}), lung absorption (32%)], and Water consumption (0.59 L day^{-1}) were maintained in the computation (US-EPA, 2010). The IEUBK model has been proven robust even when large deviations from the default parameters applied in its development are used, as has been recently demonstrated by Li et al. (2016) in the lead risk assessment of children living in polluted areas of Central China.

2.3.3. Results and Discussion

Twenty three women and seven men (n=30), with ages between 21 and 48 years old (mean: 30 ± 7), participated in the study. At the time of the study they were working or studying at the University of Aveiro and followed an omnivorous diet. Pb was detected in all the 30 duplicate diet samples with values ranging from 0.009 to 0.10 mg kg^{-1} wet weight (median 0.02 mg kg^{-1} ww). The estimated daily intakes (EDI), calculated using the Pb concentration in each sample, the individual body weight of each participant and the amount of food consumed in average by adults in Portugal in the year the sampling took place (1.867 kg inhab^{-1} day^{-1}), varied between 0.22 and 3.5 μg kg-bw^{-1} day^{-1} (median 0.47 μg kg-bw^{-1} day^{-1}).

No significant differences were obtained between the levels of Pb in the duplicate diet samples or the dietary intake of Pb and the occupation (Kruskal-Wallis test, $p > 0.05$). Additionally, no significant differences were obtained between Pb levels and the participants' gender (Mann-Whitney *U* test, $p > 0.05$). Furthermore, no significant

correlation was obtained between the levels of Pb and the participants' age (Spearman Correlation, $p > 0.05$).

Table 2.3.1 describes, to the best of our knowledge, the available duplicate diet studies (DDS) for Pb performed around the world since 2000. The mean dietary intake of Pb estimated for the present study (38.3 μg day^{-1}) approximated these determined in Italy (Alberti-Fidanza et al., 2003), India (Raghunath et al., 2006) and Germany (Wilhelm et al., 2002, 2003). A study performed in Japan indicated the lowest intake (6.74 μg day^{-1}) (Aung et al., 2006), whereas studies in Poland and China presented values above 100 μg day^{-1} (Marzec and Schlegel-Zawadzka, 2004; Liu et al., 2010). Overall, the calculated dietary intakes, by spanning three orders of magnitude (from units to hundreds μg day^{-1}) show that dietary exposure varies with geographic location. However, since some authors and international organizations demonstrated that there is no known level of Pb exposure that can be considered safe (e.g. Chiodo et al., 2007; EFSA, 2010) the exposure to Pb via diet potentially poses a worldwide health concern.

Table 2.3.1. Comparison of the Pb estimated daily intakes (EDI) means from different locations around the world.

References	Country	EDI (μg day^{-1})
Alberti-Fidanza et al. (2003)	Italy	43.1; 51.0 (♀; ♂)
Aung et al. (2006)	Japan	6.74
Domingo et al. (2012)	Spain	19.8
Liu et al. (2010)	China	102.2*
Marzec and Schlegel-Zawadzka (2004)	Poland	66.5 – 106.0 (means range)
Raghunath et al. (2006)	India	32.3 (Geo mean)
Wilhelm et al. (2002)	Germany	–
Wilhelm et al. (2003)	Germany	23.5
Present study	Portugal	38.3

*estimated considering adult weight of 70 kg.

In the present study, the median daily intake was 0.47 μg kg bw^{-1} day^{-1}. This value is very close to the dietary intakes related to the Bench Mark Dose Level (BMDL) derived from developmental neurotoxicity. Moreover, the obtained range (0.22–3.5 μg kg-bw^{-1} day^{-1}) comprises EDIs higher than the BMDLs set for the three endpoints: developmental

neurotoxicity (DN), systolic blood pressure (SBP) and chronic kidney disease (CKD) (EFSA, 2010).

In order to characterize risk associated with Pb ingestion we further applied the Margin of Exposure approach (MOE) (MOE=$BMDL_{endpoint}$/dietary exposure estimate) (EFSA, 2010). Considering that the DN endpoint is applicable solely to children, the MOE for DN was not calculated. The MOE returned a median value of 0.3 for SBP (variation between 0.2 and 2.0, but only one value above 1) and 0.8 for CKD (variation between 0.4 and 4.9, with 8 values above 1). Such results indicate that for the studied population, the risk of cardiovascular effects is reduced (3.3%), while for nephrotoxicity the effects are more significant, with a quarter of the population at risk (26.7%). This means that despite the successive measures in Portugal to reduce the release of Pb by anthropogenic activities, such as the ban of its use in the manufacture of paints and gasoline (since 1993 and 1999, respectively) (DL No. 54/93 and DL No. 186/99), the exposure to Pb is still high and raises concern relatively to possible toxic health effects.

In order to better characterize the risk associated with Pb ingestion we also calculated the blood Pb levels. The estimations of the Pb in blood for adults (Carlisle and Wade equation) are presented in Figure 2.3.1. Since Aveiro area is an important historical industrial hub, we further considered other exposure pathways (such as air, soil and dust) using local specific monitoring data (Figure 2.3.1, left side: all sources = dietary + non-dietary) (see Material and methods section for details). Calculations of the Pb blood concentrations corresponding to children following the same diet were also performed using the IEUBK model and are depicted in Figure 2.3.2.

The correspondence between dietary intakes and Pb in blood (i.e., DN: 0.50 μg kg-bw^{-1} day^{-1} = 12 μg Pb L^{-1}; SBP: 1.50 μg kg bw^{-1} day^{-1} = 36 μg Pb L^{-1}, and CKD: 0.63 μg kg-bw^{-1} day^{-1} = 15 μg Pb L^{-1}) was, as previously mentioned, calculated by the CONTAM Panel by considering only the dietary exposure fraction (see Introduction section). The panel decision was taken after due consideration of epidemiological data and health significance of observed changes associated with blood Pb levels from a considerably large amount of datasets from around the world and not necessarily representative of the European space alone.

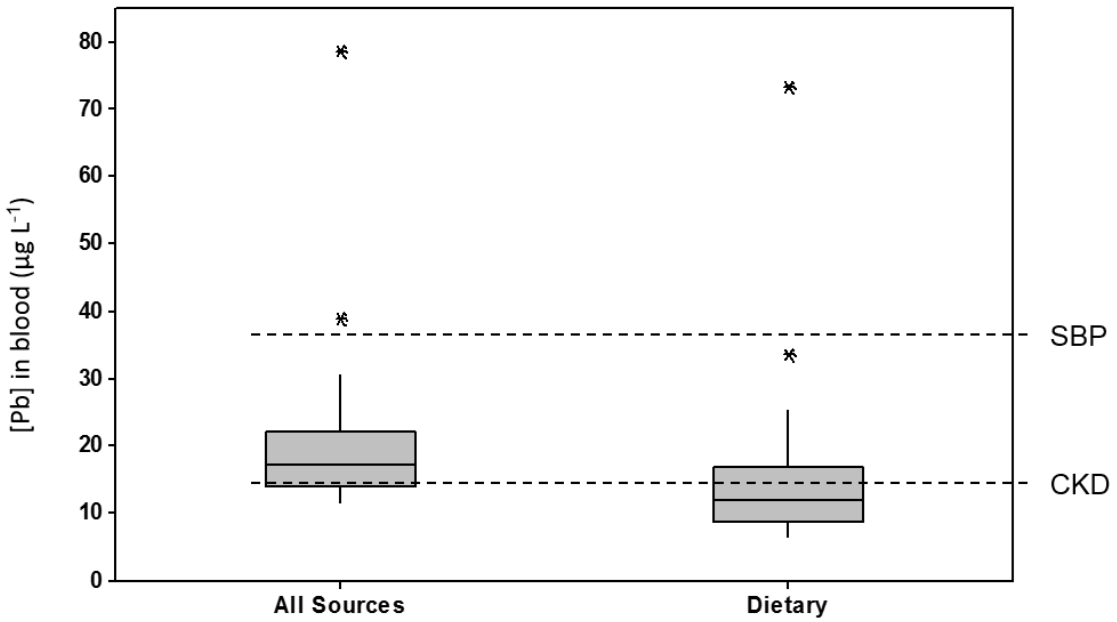

Figure 2.3.1. Estimation of Pb blood concentrations calculated using the Carlisle and Wade (1992) equation considering the diet, soil and house dust ingestion, and inhalation pathways ("All sources") and the diet pathway alone ("Dietary"). Values for elevated systolic blood pressure (SBP= 36 µg L^{-1} of blood) and chronic kidney disease (CKD= 15 µg L^{-1} of blood) endpoints as established by EFSA (2010) are indicated. Values presented are the median, 1st and 3rd quartiles, and outliers (*).

From a practical point of view, it corresponds to saying that only the dietary source can contribute to increasing blood Pb levels and thus elicit neurodevelopmental, cardiovascular and renal effects. However, as clearly indicated in the original equation (Eq. 1), all sources have a contribution to circulating Pb. The dismissal as negligible by EFSA eminently derives from a need to represent the general population and not from the lack of acknowledgement of the relevance of all sources to [Pb_B]. For this reason we used the same models to estimate Pb levels of the studied population when all sources are considered.

For adults, the dietary source corresponded to a median blood Pb concentration of 11.9 µg L^{-1}, varying between 6.5 and 73.4 µg L^{-1}. The obtained distribution is, in its majority, inside the range indicated for "average consumers" (9–30 µg L^{-1}) by EFSA (2010). However, when all sources are included in the calculation this profile becomes closer to the one indicated for "high consumers" (18–58 µg L^{-1}) (EFSA, 2010), with values raised to 17.1, 11.7 and 78.6 µg L^{-1}, respectively (Figure 2.3.1). This implies that the risk from dietary sources alone, will be raised from 3.3% for SBP and 26.7% for CKD to 6.7% for

SBP and 73.3% for CKD, when all sources are considered. Despite SBP not being in itself adverse, it is known to be linearly correlated with the incidence stroke and myocardial infarction. Chronic kidney disease is also linearly correlated with a decrease in glomerular filtration. This means that higher concentrations of circulating Pb in blood will correspond to higher blood pressure and lower glomerular filtration rates, which increases the possibility of effects in some participants. The differences obtained between the two calculation approaches (diet alone versus all sources) reinforces the need to use all sources when evaluating risk associated with lead exposure.

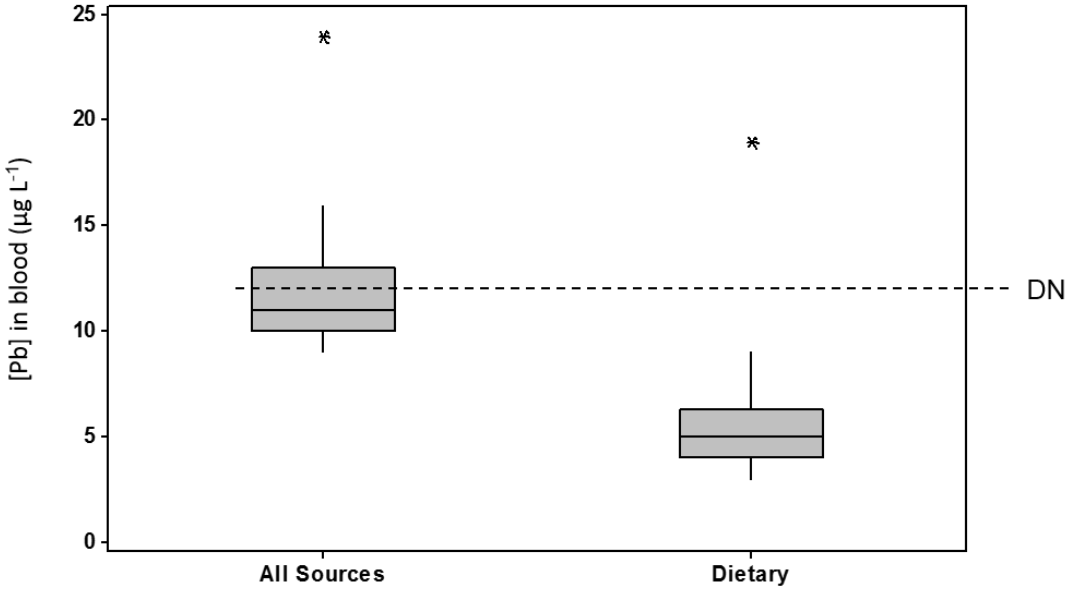

Figure 2.3.2. Estimation of Pb blood concentrations in children between 6 and 7 years old hypothetically following the same diet as the participants in this study calculated using the USEPA's IEUBK model (IEUBKwin v1.1 build 11) (USEPA, 2010), considering the inhalation, diet, drinking water, and soil and house dust ingestion pathways ("All sources") and the diet pathway alone ("Dietary"). The Value for developmental neurotoxicity endpoint (DN= 12 µg L^{-1} of blood) is indicated. Values presented are the median, 1st and 3rd quartiles, and outliers (*).

The estimations of the [Pb] in children's blood demonstrated the same patterns: when only the dietary contribution is considered the obtained median is 5.0 µg L^{-1} (varying between 3.0 and 19 µg L^{-1}) corresponding to an estimated 3.3% risk, whereas when all sources are summed these values increase to a median of 11.0 µg L^{-1} (a variation of 9.0 to 24.0 µg L^{-1}) and a developmental neurotoxicity risk of 30%. This comes as a corroboration of the importance of considering all sources of exposure. However, both estimations of [Pb_B] are within the range of values considered by EFSA (2010) for an "average consumer" (15–46

μg L^{-1}) for children between 4 and 7 years, which is in line with the conclusion of the CONTAM Panel that children in this range might be suffering effects. It should be noted that the EFSA dietary intakes were calculated based on an average 12.5 kg child whereas our calculation was based on the average of the boys and girls 50th percentile for Portuguese 7 year olds (=21.5 kg) (DGS, 2006) consequently yielding lower intakes when normalized to body weight.

2.3.4. Conclusions

This study analysed the levels of Pb in duplicate diet samples, and therefore it gives information on the exposure scenario very similar to the reality, since duplicate diet samples allow the quantification of the actual levels to which the participants are exposed. Pb was detected in the 30 analysed duplicate diet samples; therefore, all participants were exposed to this toxic metal through the ingestion of food. Alongside with the fact that there is no safe limit for Pb our results disclose that cardiovascular and nephrotoxic effects will likely occur in at least 3.3 and 26.7% of the volunteers**.**

Acknowledgments

The authors wish to deeply acknowledge the volunteers who participated in this study and provided for 7 consecutive days the duplicates of their diet. The assistance of Adi S. Riyadi and Toshiki Kamei during chemical analysis is deeply acknowledged. This work was supported by European Funds through COMPETE and by National Funds through the Portuguese Science Foundation (FCT) within projects PEst-C/MAR/LA0017/2013 and PEst-OE/SAU/UI0709/2014. Ana C. A. Sousa and Sónia D. Coelho acknowledge FCT for the grants SFRH/BPD/65884/2009 and SFRH/ BD/78168/2011 (supported by funding from the Human Potential Operational Programme POPH, inscribed in the National Strategic Reference Framework and partially subsidized by the European Social Fund). This study was partly supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) to a project on Joint Usage/Research Center – Leading Academia in Marine and Environmental Research (LaMer), Ehime University. Further support was provided by Grants-in-Aid for Scientific Research (A: 25257403 and 16H01784) of Japan Society for the Promotion of Science (JSPS).

References

ADRA, 2014. Águas da Região de Aveiro, Zona de Abastecimento AVR IV. http://www.adra.pt/content/index.php?action=detailfo&rec=1924&t=Qualidade-da-Agua-na-Zona-de-Abastecimento-AVR-IV. (accessed March, 2016)

Alberti-Fidanza, A., Burini, G., Perriello, G., Fidanza, F., 2003. Trace element intake and status of Italian subjects living in the Gubbio area. Environ. Res. 91, 71-77.

ATSDR, 2007. Toxicological profile for Lead. Public Health Service Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services.

Aung, N.N., Yoshinaga, J., Takahashi, J.I., 2006. Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan. Food Addit. Contam. 23, 883-894.

Carlisle, J.C., Wade, M.J., 1992. Predicting blood lead concentrations from environmental concentrations. Regul. Toxicol. Pharm. 16, 280-289.

Chaney, R.L., Mielke, H.W., Sterrett, S.B., 1989. Speciation, mobility, and bioavailability of Soil Lead. [Proc. Intern. Conf. Lead in Soils: Issues and Guidelines. B.E. Davies & B.G. Wixson (eds.)]. Environ. Geochem. Health 11, 105-129.

Chiodo L.M., Covington C., Sokol R.J., Hannigan J.H., Jannise J., Ager J., Greenwald M., Delaney-Black V., 2007. Blood lead levels and specific attention effects in young children. Neurotoxicol.Teratol. 29, 538-546

Coelho S.D., Sousa A.C.A., Isobe T., Kunisue T., Nogueira A.J.A., Tanabe S., 2016. Brominated flame retardants and organochlorine compounds in duplicate diet samples from a Portuguese academic community. Chemosphere 160, 89-94.

DGS, 2006. Direção Geral da Saúde. Divisão de Saúde Materna, Infantil e dos Adolescentes. Circular Normativa Nº: 05/DSMIA: Actualização das Curvas de Crescimento.

DGS, 2014. Direção Geral da Saúde. Direção de Serviços de Informação e Análise Portugal. Programa Nacional para a Promoção da Alimentação Saudável.

Domingo, J., Perelló, G., Giné Bordonaba, J., 2012. Dietary Intake of Metals by the Population of Tarragona County (Catalonia, Spain): Results from a Duplicate Diet Study. Biol. Trace Elem. Res. 146, 420-425.

EFSA, 2010. Scientific Opinion on Lead in Food. EFSA J. 151.

EFSA, 2012. Lead dietary exposure in the European population. EFSA J. 59.

Freitas, M.C., Farinha, M.M., Ventura, M.G., Almeida, S.M., Reis, M.A., Pacheco, A.M.G., 2005. Gravimetric and Chemical Features of Airborne PM10 AND PM2.5 in Mainland Portugal. Environ. Monit. Assess. 109, 81-95.

IARC, 2006. Inorganic and organic lead compounds. IARC Monographs on the evaluation of carcinogenic risks to human, pp. 519.

INE, 2014. Balança Alimentar Portuguesa 2008-2012. Destaque - Informação à Comunicação, pp. 1-25.

Li Y., Hu J., Wu W., Liu S., Li M., Yao N., Chen J., Ye L., Wang Q., Zhou Y., 2016. Application of IEUBK model in lead risk assessment of children aged 61–84 months old in central China. Sci. Total Environ. 541, 673-682

Liu, P., Wang, C.-N., Song, X.-Y., Wu, Y.-N., 2010. Dietary intake of lead and cadmium by children and adults – Result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method. Int. J. Hyg. Environ. Health 213, 450-457.

Marzec, Z., Schlegel-Zawadzka, M., 2004. Exposure to cadmium, lead and mercury in the adult population from Eastern Poland, 1990-2002. Food Addit. Contam. 21, 963-970.

Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T., 2007. Chapter 1 - Introduction—General Considerations and International Perspectives. in: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.). Handbook on the Toxicology of Metals (Third Edition). Academic Press, Burlington, pp. 1-9.

Patinha, C., Reis, A.P., Dias, C., Cachada, A., Adao, R., Martins, H., Ferreira da Silva, E., Sousa, A.J., 2012. Lead availability in soils from Portugal's Centre Region with special reference to bioaccessibility. Environ. Geochem. Health 34, 213-227.

Pavón, J.M.C., de Torres, A.G., Rojas, F.S., Ojeda, C.B., Alonso, E.V., 2015. The toxic elements. in: de la Guardia, M., Garrigues, S. (Eds.). Handbook of Mineral Elements in Food. John Wiley & Sons, Ltd, pp. 123-152.

Raghunath, R., Tripathi, R.M., Suseela, B., Bhalke, S., Shukla, V.K., Puranik, V.D., 2006. Dietary intake of metals by Mumbai adult population. Sci. Total. Environ. 356, 62-68.

Roosens L., Abdallah MA-E., Harrad S., Neels H., Covaci A., 2009. Factors Influencing Concentrations of Polybrominated Diphenyl Ethers (PBDEs) in Students from Antwerp, Belgium. Environ. Sci. Technol. 43, 3535-3541.

Sousa, A., Pastorinho, M., Itai, T., Takahashi, S., Tanabe, S., Taborda-Barata, L., Nogueira, A., 2012. Lead concentrations in urban house dust from Portugal. 9th International Symposium on Environmental Geochemistry, July 15-21, Aveiro, Portugal, p. 45.

US-EPA, 1986. Air Quality Criteria for Lead (Final, 1986). U.S. Environmental Protection Agency, Washington, D.C.

US-EPA, 2007. EPA 9285.7-42: User's Guide for the Integrated Exposure Uptake Biokinetic Model for Lead in Children (IEUBK).

US-EPA, 2008. OSWER 9285.7-81: Guidance for The Sampling And Analysis of Lead in Indoor Residential Dust for use in The Integrated Exposure Uptake Biokinetic (IEUBK) Model.

US-EPA, 2010. Integrated Exposure Uptake Biokinetic Model for Lead in Children, Windows® version (IEUBKwin v1.1 build 11).

Wilhelm, M., Wittsiepe, J., Schrey, P., Budde, U., Idel, H., 2002. Dietary intake of cadmium by children and adults from Germany using duplicate portion sampling. Sci. Total. Environ. 285, 11-19.

Wilhelm, M., Wittsiepe, J., Schrey, P., Feldmann, C., Idel, H., 2003. Dietary intake of lead by children and adults from Germany measured by the duplicate method. Int. J. Hyg. Environ. Health 206, 493-503.

2.4. Cadmium intake in women from the University of Aveiro, Portugal – a duplicate diet study

Abstract

Cadmium (Cd) is a non-essential metal widespread in the environment, to which humans are exposed through different routes, being food consumption the most important one. It is considered an endocrine disruptor that is associated with estrogen-dependent diseases with women being particularly susceptible. In order to assess the exposure to this metal through diet intake in premenopausal women, levels of Cd were quantified in 7-days duplicate diet samples provided by 23 women working or studying at University of Aveiro, Portugal. Cd was detected in all analyzed samples with concentrations ranging between 0.007 and 0.21 μg g^{-1} ww (median: 0.009 μg g^{-1} ww). The estimated dietary weekly intakes varied from 1.4 to 48 μg kg-bw^{-1} $week^{-1}$ (median: 2.3 μg kg-bw^{-1} $week^{-1}$), and 35% of the participants exhibited dietary intakes of Cd higher than the tolerable weekly intake (2.5 μg kg-bw^{-1} $week^{-1}$) set for this metal which suggest health risks for these women.

Keywords: cadmium; diet; women exposure; Portugal.

2.4.1. Introduction

Cadmium (Cd) is a non-essential metal that occurs in the environment naturally or as the result of humans activities, which are considered responsible for the increasing of Cd environmental levels over the past years (WHO, 2010). The main anthropogenic Cd sources include mining, fossil fuel combustion, waste incineration, manufacture of phosphate fertilizers and tobacco smoking (Nordberg et al., 2007). Since the 90s that the use of Cd decreased considerably in the European Union as a consequence of the reduction in the manufacture of nickel-cadmium batteries and other tools and with the implementation of more restrictive legislation, such as the Directive 91/338/EEC, an amendment of Directive 76/769/EEC concerning the restrictions on the marketing and use of certain dangerous substances and preparations, adding Cd to this list.

The general population can be exposed through the ingestion of contaminated food, soil and dust, drinking water or from the inhalation of contaminated particles from ambient air or cigarette smoke (NIEHS, 2011; ATSDR, 2012). The consumption of food is the major pathway of human exposure, accounting for about 90% of the total intake in the non-smoking general population (EFSA, 2012).

Cd is associated with several adverse effects on human health even when exposure occurs at low levels, mainly because it efficiently accumulates in the kidney and liver and has a long biological half-life (from 10 to 30 years) (Verougstraete et al., 2003; EFSA, 2012). It affects primarily the kidney, but also the skeletal and respiratory systems (WHO, 2010) and it is classified as a ”human carcinogen” (Group 1) (IARC, 2012). According to several *in vitro* and animal studies, Cd is an estrogenic endocrine disruptor (Gore et al., 2015). It binds to estrogen receptors and mimics the effects of estradiol, activating the estrogen signalling pathways (Stoica et al., 2000; Liu et al., 2008). This might lead to an increased risk of estrogen-dependent diseases, such as endometriosis, breast, endometrial and ovarian cancer (Garcia-Morales et al., 1994; Johnson et al., 2003; Jackson et al., 2008; Adams et al., 2014; Itoh et al., 2014) and therefore women are considered a susceptible group.

Considering that the ingestion of food is one of the major pathways of exposure to Cd, and given the known associations of Cd exposure with women’s health, the aim of the present study was to characterize the exposure of women from an academic community

(University of Aveiro) to Cd through diet, by evaluating its levels in duplicate diet samples.

2.4.2. Material and Methods

2.4.2.1. Sampling

In order to estimate the exposure to several classes of environmental contaminants through food, a duplicate diet study (DDS) was launched at University of Aveiro in 2012. Duplicate diet studies allow estimating the real exposure through food being a cost effective method when compared to total diet studies. Furthermore, DDS are considered more adequate than estimations through dietary record or food frequency questionnaires, which can over or underestimate the real contaminants intake (Shim et al., 2014). Details on the sampling campaign and results on the levels of brominated flame retardants, organochlorine compounds, organotin compounds and lead were recently published elsewhere (Coelho et al., (2016a); Coelho et al., (2016b); Sousa et al., (2017)). In brief, each sample contained a duplicate portion representative of all the food items (excluding beverages) consumed by each participant during 7 consecutive days. From the obtained set of samples those provided by women were selected (n=23) and analysed.

2.4.2.2. Chemical analysis

About 200 mg of each freeze dried sample was digested with concentrated nitric acid (Wako Pure Chemical Industries, Ltd.) in a microwave system (Ethos D, Milestone). Cadmium (Cd) levels were quantified by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500cx, Agilent Technologies).

For QA/QC, certified calibration standards (Wako Pure Chemical Industries, Japan) were used. Instrumental drift was calibrated using Rhodium as internal standard. With each batch of samples, procedure blanks were performed and a certified standard reference material (*oyster tissue* - NIST SRM 1566b) was analysed. Recoveries evaluated by SRM 1566b varied between 100 and 104%.

2.4.2.3. Dietary intake calculation

The estimation of cadmium daily intakes (EDI) and weekly intakes (EWI) was based on the concentrations of Cd in each sample, the individual weight of the volunteers' (data retrieved from the questionnaire) and the per capita daily food consumption of 1867.2 g inhab^{-1} day^{-1} set by the National Institute of Statistics, Portugal (INE, 2014).

2.4.3. Results and Discussion

2.4.3.1. Cadmium concentrations and dietary intakes

Twenty three women participated in the study, with ages ranging between 21 and 42 years and body weights from 45 to 70 kg. Cd was detected in all samples demonstrating widespread occurrence in the diet of the studied women, with concentrations varying from 0.007 to 0.21 µg g^{-1} ww (Table 2.4.1).

Table 2.4.1. Concentrations of Cd in the 23 duplicate diet samples, estimated dietary daily intakes and respective tolerable weekly/daily intakes.

	Concentrations (µg g^{-1} ww)	**Daily intake - diet** (µg day^{-1}) (µg kg-bw^{-1} day^{-1})	**Weekly intake - diet** (µg kg-bw^{-1} week^{-1})	**Tolerable intake** (µg kg-bw^{-1} week^{-1})
Mean	0.022	42 0.73	5.1	
Median	0.009	17 0.32	2.3	2.5[a]
Range	0.007 – 0.21	12 – 400 0.20 – 6.8	1.4 – 48	

Estimated daily intakes were calculated considering 1867.2 g inhab^{-1} day^{-1} as the daily edible per capita in 2012 (INE, 2014) and the participants body weights reported in the food frequency questionnaires.
[a] EFSA (2011).

The estimated daily intakes (EDIs) ranged between 0.2 and 6.8 µg kg-bw^{-1} day^{-1} (median: 0.32 µg kg-bw^{-1} day^{-1}, mean 0.73 µg kg-bw^{-1} day^{-1}). Converting to weekly intake, the obtained value of 2.3 µg kg-bw^{-1} week^{-1} was very close to the Tolerable Weekly Intake (TWI) of 2.5 µg kg-bw^{-1} week^{-1} established by the Panel on Contaminants in the Food Chain of the European Food Safety Authority (CONTAM Panel - EFSA) in 2011 (EFSA, 2011). Although the median intake was below the tolerable intake, 35% of the women who

participated in this study exhibited EWIs higher than the established TWI (2.5 µg kg-bw^{-1} week^{-1}). Furthermore, one sample exhibited extremely high values of Cd and consequently the EWI for this sample was very high (6.8 µg kg-bw^{-1} day^{-1}).

2.4.3.2. Comparison with other studies

The estimated daily intakes of Cd in this study and the ones obtained from other duplicate diet studies are shown in Figure 2.4.1. Overall, great variations between surveys can be observed. These differences between studies, are probably due to the differences in the diet adopted in each country, as the amount of Cd varies significantly among different food items (Adams et al., 2012), and therefore the composition of the duplicate diet samples also varies. Portugal (present study) and Spain (Domingo et al., 2012) stand out with highest mean daily intakes (42 and 49.5 µg day^{-1}, respectively), which are higher than the established tolerable intake. This is probably due to the fact that both countries follow the Mediterranean Diet (INE, 2014) in which a higher amount of vegetables known to be important sources of Cd (IARC, 2012), are consumed. However, for Italy, which also follows the Mediterranean diet, the mean intakes of Cd were much lower (7.0 µg day^{-1}) (Alberti-Fidanza et al., 2003). Such results might be due to the differences in the sampling period and method. In the Italian study, duplicate diet samples were collected only for 2 days whilst in the Spanish and Portuguese surveys duplicate diet samples were collected for 10 and 7-days, respectively. Therefore, the samples' composition between the Italian and the Iberian studies were different with the Spanish and Portuguese samples being more diverse, representative and, consequently closer to reality as a result of the longer sampling periods.

The majority of the studies (Figure 2.4.1), some with robust datasets (Wilhelm et al., 2002; Raghunath et al., 2006; Domingo et al., 2012), disclosed a significant fraction of results above the tolerable daily intake (dashed line) established by EFSA. As aforementioned, these results demonstrate that the exposure to Cd through the ingestion of food poses risks as the levels of this metal in duplicate diet samples from different countries are higher than the highest level set to ensure the protection of consumers through diet alone (Figure 2.4.1). Furthermore, the real exposure to Cd and related health hazards will be enhanced if all the exposure pathways are considered.

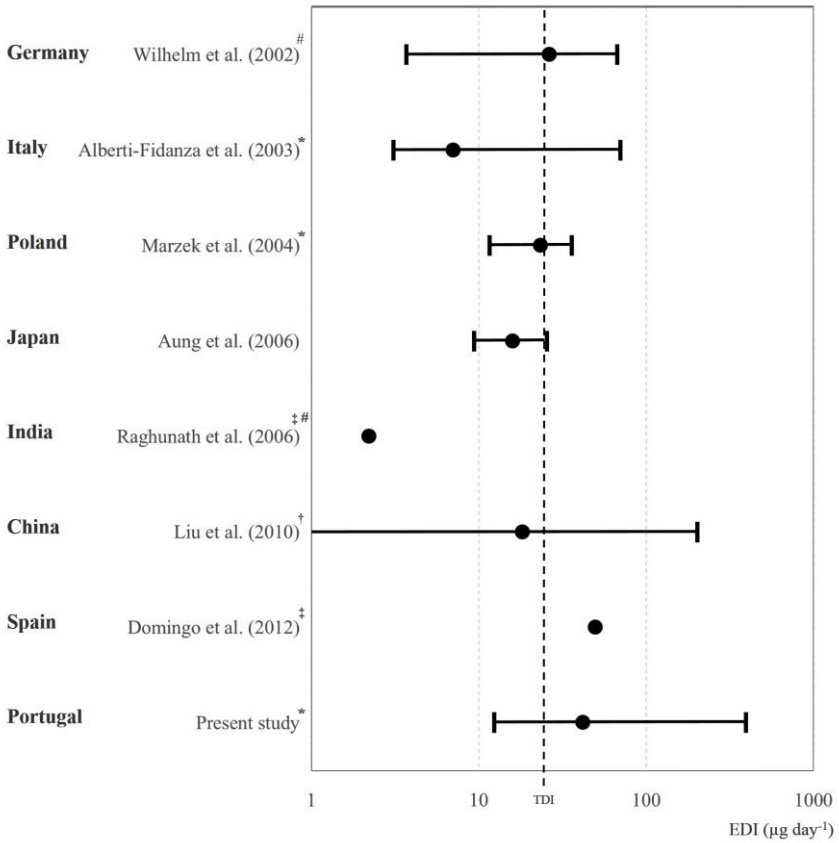

Figure 2.4.1. Comparison of Cd Estimated Daily Intakes (EDI) calculated from duplicate diet studies (range, mean (dot)). Dashed line – represents the tolerable daily intake (TDI) set by EFSA adjusted by dividing the tolerable weekly intake by 7 and considering a body weight of 70 Kg. #Geometric mean. *Only women included. ‡Only mean values available. †EDI adjusted for a body weight of 70 kg. (Logarithmic scale was used).

Breast cancer is the leading cause of cancer death among women (Jemal et al., 2011) and over the last years several reports on the possible associations with dietary Cd intake have been published. However, they disclosed conflicting results, with some studies revealing positive associations whereas others could not find evidences of an association of dietary Cd with this type of cancer. Adams at al. (2014), for example, found little evidence that dietary Cd is a risk factor for breast cancer in a large and well-characterized group from the Women's Health Initiative study (with over 6,000 women with breast cancer). On the contrary, a positive association between dietary Cd and risk of postmenopausal breast cancer was obtained in a large cohort study in Sweden (Swedish Mammography Cohort study; n= 55,987 postmenopausal women (Julin et al., 2012)). Recently, the association between Cd and breast cancer was reviewed in two different meta-analysis (Lin et al., 2016; Van Maele-Fabry et al., 2016). Overall, the reports selected for the meta-analysis

used an indirect method to estimate the Cd daily intake (self-administered food frequency questionnaire), which may miscalculate the exposure. Furthermore, in the majority of these studies the exposure to Cd was assessed by means of urinary Cd concentrations which reflect not only Cd intake through multiple sources, but also past exposures (given the long half-life of this metal), and therefore cumulative exposures, and diet based estimations should constitute standard operational procedure in future studies. Additionally, the differences in individual susceptibility to Cd effects between women might also be a bias (Rentschler et al., 2014), and adjustments to several confounders also need to be considered in future works.

2.4.4. Conclusions

To the best of our knowledge, this is the first study in Portugal assessing the Cd dietary intake in women using a duplicate diet method, allowing the quantification of the actual levels to which these women were exposed through the consumption of food. Our results disclose that all women were exposed to Cd through this pathway, confirming diet as an important source of Cd. In 35% of the cases the estimated dietary intakes of Cd were higher than the established tolerable intake suggesting an increased health risk for these premenopausal women.

Acknowledgments

The authors wish to deeply acknowledge the women who participated in this study. This work was supported by European Funds through COMPETE and by National Funds through the Portuguese Science Foundation (FCT) within projects PEst-C/MAR/LA0017/2013 and PEst-OE/SAU/UI0709/2014 and the grants SFRH/BPD/65884/2009 and SFRH/ BD/78168/2011 (supported by funding from the Human Potential Operational Programme POPH, inscribed in the National Strategic Reference Framework and partially subsidized by the European Social Fund). This study was partly supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) to a project on Joint Usage/Research Center – Leading Academia in Marine and Environmental Research (LaMer), Ehime University. Further support was provided by Grants-in-Aid for Scientific Research (A: 25257403 and 16H01784) of Japan Society for the Promotion of Science (JSPS).

References

Adams, S.V., Newcomb, P.A., White, E., 2012. Dietary cadmium and risk of invasive postmenopausal breast cancer in the VITAL cohort. Cancer Causes Control 23, 845-854.

Adams, S.V., Quraishi, S.M., Shafer, M.M., Passarelli, M.N., Freney, E.P., Chlebowski, R.T., Luo, J., Meliker, J.R., Mu, L., Neuhouser, M.L., Newcomb, P.A., 2014. Dietary Cadmium Exposure and Risk of Breast, Endometrial, and Ovarian Cancer in the Women's Health Initiative. Environ. Health Persp. 122, 594-600.

Alberti-Fidanza, A., Burini, G., Perriello, G., Fidanza, F., 2003. Trace element intake and status of Italian subjects living in the Gubbio area. Environ. Res. 91, 71-77.

ATSDR, 2012. Public Health Statement - Cadmium. Agency for Toxic Substances and Disease Registry. Department of Health And Human Services, Public Health Service.

Aung, N.N., Yoshinaga, J., Takahashi, J.I., 2006. Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan. Food Addit. Contam. 23, 883-894.

Coelho, S.D., Sousa, A.C.A., Isobe, T., Kunisue, T., Nogueira, A.J.A., Tanabe, S., (2016a). Brominated flame retardants and organochlorine compounds in duplicate diet samples from a Portuguese academic community. Chemosphere 160, 89-94.

Coelho, S.D., Pastorinho, M.R., Itai, T., Isobe, T., Kunisue, T., Nogueira, A.J.A., Tanabe, S., Sousa, A.C.A., (2016b). Lead in duplicate diet samples from an academic community. Sci. Total. Environ. 573, 603-607.

Domingo, J.L., Perello, G., Gine Bordonaba, J., 2012. Dietary intake of metals by the population of Tarragona County (Catalonia, Spain): results from a duplicate diet study. Biol. Trace Elem. Res. 146, 420-425.

EFSA, 2011. Statement on tolerable weekly intake for cadmium. EFSA J. 19.

EFSA, 2012. Cadmium dietary exposure in the European population. EFSA J. 37.

Garcia-Morales, P., Saceda, M., Kenney, N., Kim, N., Salomon, D.S., Gottardis, M.M., Solomon, H.B., Sholler, P.F., Jordan, V.C., Martin, M.B., 1994. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. J. Biol. Chem. 269, 16896-16901.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr. Rev. 36, E1-E150.

IARC, 2012. Cadmium and Cadmium Compounds. IARC monographs on the evaluation of carcinogenic risks to humans, 26 pp.

INE, 2014. Balança Alimentar Portuguesa 2008-2012. Destaque - Informação à Comunicação, 1-25.

Itoh, H., Iwasaki, M., Sawada, N., Takachi, R., Kasuga, Y., Yokoyama, S., Onuma, H., Nishimura, H., Kusama, R., Yokoyama, K., Tsugane, S., 2014. Dietary cadmium intake and breast cancer risk in Japanese women: A case-control study. Int. J. Hyg. Environ. Health 217, 70-77.

Jackson, L.W., Zullo, M.D., Goldberg, J.M., 2008. The association between heavy metals, endometriosis and uterine myomas among premenopausal women: National Health and Nutrition Examination Survey 1999–2002. Hum. Reprod. 23, 679-687.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E., Forman, D., 2011. Global cancer statistics. CA Cancer J. Clin. 61, 69-90.

Johnson, M.D., Kenney, N., Stoica, A., Hilakivi-Clarke, L., Singh, B., Chepko, G., Clarke, R., Sholler, P.F., Lirio, A.A., Foss, C., Reiter, R., Trock, B., Paik, S., Martin, M.B., 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. Nat. Med. 9, 1081-1084.

Julin, B., Wolk, A., Bergkvist, L., Bottai, M., Akesson, A., 2012. Dietary cadmium exposure and risk of postmenopausal breast cancer: a population-based prospective cohort study. Cancer Res. 72, 1459-1466.

Lin, J., Zhang, F., Lei, Y., 2016. Dietary intake and urinary level of cadmium and breast cancer risk: A meta-analysis. Cancer Epidemiol. 42, 101-107.

Liu, P., Wang, C.-N., Song, X.-Y., Wu, Y.-N., 2010. Dietary intake of lead and cadmium by children and adults – Result calculated from dietary recall and available lead/cadmium level in food in comparison to result from food duplicate diet method. Int. J. Hyg. Environ. Health 213, 450-457.

Liu, Z., Yu, X., Shaikh, Z.A., 2008. Rapid activation of ERK1/2 and AKT in human breast cancer cells by cadmium. Toxicol. Appl. Pharm. 228, 286-294.

Marzec, Z., Schlegel-Zawadzka, M., 2004. Exposure to cadmium, lead and mercury in the adult population from Eastern Poland, 1990--2002. Food Addit. Contam. 21, 963-970.

NIEHS, 2011. 12th Report on Carcinogens, 499 pp.

Nordberg, G.F., Nogawa, K., Nordberg, M., Friberg, L.T., 2007. Chapter 23 - Cadmium. in: Nordberg, G.F., Fowler, B.A., Nordberg, M., Friberg, L.T. (Eds.). Handbook on the Toxicology of Metals (Third Edition). Academic Press, Burlington, pp. 445-486.

Raghunath, R., Tripathi, R.M., Suseela, B., Bhalke, S., Shukla, V.K., Puranik, V.D., 2006. Dietary intake of metals by Mumbai adult population. Sci. Total. Environ. 356, 62-68.

Rentschler, G., Kippler, M., Axmon, A., Raqib, R., Skerfving, S., Vahter, M., Broberg, K., 2014. Cadmium concentrations in human blood and urine are associated with polymorphisms in zinc transporter genes. Metallomics 6, 885-891.

Shim, J.-S., Oh, K., Kim, H.C., 2014. Dietary assessment methods in epidemiologic studies. Epidemiol. Health 36, e2014009.

Sousa, A.C.A., Coelho, S.D., Pastorinho, M.R., Taborda-Barata, L., Nogueira, A.J.A., Isobe, T., Kunisue, T., Takahashi, S., Tanabe, S., (2017). Levels of TBT and other selected organotin compounds in duplicate diet samples. Sci. Total. Environ. 574, 19-23.

Stoica, A., Katzenellenbogen, B.S., Martin, M.B., 2000. Activation of estrogen receptor-alpha by the heavy metal cadmium. Mol. Endocrinol. 14, 545-553.

Van Maele-Fabry, G., Lombaert, N., Lison, D., 2016. Dietary exposure to cadmium and risk of breast cancer in postmenopausal women: A systematic review and meta-analysis. Environ. Int. 86, 1-13.

Verougstraete, V., Lison, D., Hotz, P., 2003. Cadmium, Lung and Prostate Cancer: A Systematic Review of Recent Epidemiological Data. J. Toxicol. Environ. Health B. 6, 227-256.

WHO, 2010. Exposure to cadmium A major public health concern. Public Health and Envionment - World Health Organization, 4 pp.

Wilhelm, M., Wittsiepe, J., Schrey, P., Budde, U., Idel, H., 2002. Dietary intake of cadmium by children and adults from Germany using duplicate portion sampling. Sci. Total. Environ. 285, 11-19.

CHAPTER 3

HOUSE DUST

3.1. Flame retardants in indoor dust - a review on the levels of polybrominated diphenyl ethers and hexabromocyclododecanes

Abstract

Modern people spend a considerable time of their life indoors, whether at home, at the workplace or at school, or inside vehicles and public transportation, therefore exposure to a variety of contaminants present indoors is constant and profuse. These contaminants released from household products tend to accumulate and concentrate in dust which is thus considered as one of the main human exposure pathways to several chemicals either by inhalation or ingestion. Within this wide range of contaminants polybrominated diphenyl ethers and hexabromocyclododecanes are included. These two brominated flame retardants have been applied in a vast range of materials with the aim to inhibit or delay the combustion and prevent the fire progression thus increasing the available time for people to escape. The extensive usage granted them a ubiquitous presence in the indoor environment and also in humans. Due to their toxicity and their potential to bioaccumulate these flame retardants have been restricted or banned. However, their persistency in the environment and the increasing evidences of deleterious effects towards humans and wildlife renders the study of these contaminants a matter of great importance.

In this review we gathered available information on the levels of PBDEs and HBCDDs in indoor dust samples collected from different places and different regions around the world and discuss human exposure to these contaminants through dust.

Keywords: flame retardants, hexabromocyclododecanes, human exposure, indoor dust, polybrominated diphenyl ethers.

3.1.1. Introduction

With the exponential growth of the chemical industry the use of synthetic chemicals become massive and therefore humans are continuously exposed to such substances (Meeker, 2012) particularly in the indoor environment, where people tend to spend the vast majority of their life time. Hence, daily human exposure to these harmful chemicals has become, in the last decades, one of the major concerns to the public as well as the scientific community. This concern emerged as a result of a large number of studies that revealed that some of these compounds have the ability to interfere with any aspect of hormone action in humans and wildlife (Zoeller et al., 2012). These pollutants, classified as endocrine-disrupting chemicals (EDCs), used in a wide range of products and goods, pose increased risks towards human health (WHO/UNEP, 2013), rendering them to a matter of great concern.

It is well established that contaminants are globally distributed and the number of sources is vast (WHO/UNEP, 2013). The main routes of human contact to these contaminants are air, soil, water, food and dust (Lioy et al., 2002). Indoor air and dust have been used for the last decades as essential elements for persistent chemicals monitoring as people in modern societies tend to spend over 80% of time indoors (Butt et al., 2004; Le Cann et al 2011). As the quality of the indoor environment is an increasing and global public health concern, indoor dust has been widely used to assess the exposure of humans to a diverse number of indoor environmental contaminants.

Indoor dust is a complex mixture composed of organic and inorganic matter like pollen, fungi, animal fibers, furniture and carpet fibers, interior house paint and street dust amongst others (Lioy et al., 2002; Hwang et al., 2008). It works as a receptor and repository for various environmental chemical pollutants (Mercier et al., 2011) brought from outdoors or released from products inside the house. Once accumulated in settled dust, chemicals are available for humans through ingestion and inhalation of resuspended particles and dermal absorption (Whitehead et al., 2011; Dodson et al., 2012), especially for toddlers who spend large part of their day crawling or playing on the floor repeating the hand to mouth behavior several times (Figure 3.1.1).

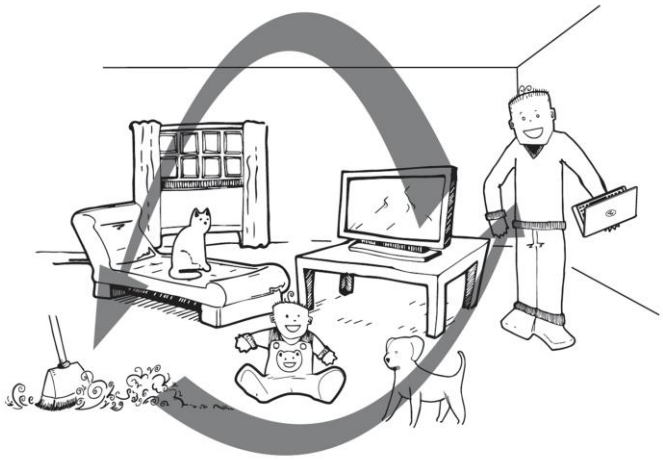

Figure 3.1.1. Schematic representation of sources and pathways of flame retardants in the indoor environment. ©Jorge Dias

Amongst the large number of indoor contaminants already detected and reported by several publications, the flame retardants (FRs) are a class of chemicals that have adverse effects on human health (Shaw et al., 2010). FRs have been applied for several decades in consumer and industrial products used in our everyday life, such as plastics, textiles and electronic equipment (Papachlimitzou et al., 2012) to slow the combustion of resins and polymers preventing fires and increasing the available time for people to escape (Marklund et al., 2003). Besides preventing flames and thus reducing the associated risk, they are economically favorable by reducing the cost of fires (Birnbaum and Staskal, 2004). In 2011, the global market sale of FRs was about $4 billion per annum, and it is expected to grow to $5.8 billion by 2018 (Ceresana, 2011). In 2013, for instance, more than 2 million tons were consumed worldwide (Ceresana, 2014).

Flame retardants include many classes of compounds such as inorganic, organophosphorus and organohalogenated FRs. Halogenated FRs can be divided into chlorinated and brominated flame retardants (BFRs), the latter being the target compounds of this review. BFRs constitute a large part of the total flame retardants production; in 2005 approximately 311 000 metric tons were used worldwide and this global market volume increased to 410 000 tons in 2008 (Shoeib et al., 2012) Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDDs) and tetrabromobisphenol-A (TBBPA) are examples for BFRs. Polybrominated diphenyl ethers, used mainly in plastics, textiles, electronics and printed circuit boards (EFSA, 2011b) are considered neurotoxic and their

endocrine disrupting potential has been confirmed by animal toxicological and risk assessment data (Allen et al., 2008; Besis et al., 2012). Hexabromocyclododecanes is associated with the disruption of thyroid hormones and is known to induce cancer in humans, among other negative effects (Covaci et al., 2006). Its main uses include thermal insulation in the building industry. Tetrabromobisphenol-A is mostly applied in printed circuit boards and thermoplastics, and it is also an endocrine disruptor (Covaci et al., 2009).

PBDEs were, until recently, the most widely applied FRs, but in the past several years they have been banned in many countries due to their persistence, bioaccumulation, long-range transport and adverse health effects (Meeker et al., 2010; Kim et al., 2011b). Considering such restrictions, a new group of BFRs began to be commercialized in order to replace the discontinued PBDEs, the "novel" brominated flame retardants (NBFRs) which have been rising in the market, being used in a large variety of products such as plastics, foams and textiles (Papachlimitzou et al., 2012). The group definition is attributed considering all the BFRs that are new in the market or recently detected in the environment (Covaci et al., 2011). Toxicological data is very limited at present.

Despite the restrictions on the use of PBDEs and HBCDDs they are still widespread because these contaminants are persistent and bioaccumulative (Covaci et al., 2011) remaining present in the environment for a long time. Even after ban on production, industrial/household products containing BFRs are used for long period and chemicals would be released to the environment during their usage and disposal/recycle processes. This fact, together with its previous ubiquitous usage, justifies continuous studies on the levels and possible health effects. Regarding the "novel" brominated flame retardants, they became an important study issue in recent years because of their extensive production and usage that led to an increase of their levels in the environment.

The aims of this review were to describe the levels of PBDEs and HBCDDs in indoor dust samples collected from different places and different regions around the world and to discuss human exposure to these contaminants through dust. All the information was obtained from a search performed on September 2013 in the Scopus bibliographic database using the keywords "dust" and "flame retardants". All the publications published between 2003 and 2013 related to indoor dust and BFRs (PBDEs and HBCDDs,) were considered and additional papers were selected from the references in the retrieved publications. Data

concerning BFRs concentrations in houses located close to electronic waste centers (high exposure scenarios) and data from studies conducted in airplanes (occasional exposure) were not included as we are interested in discussing normal exposure scenarios.

Since the statistical measures (e.g., median, mean, maximum values, minimum values) used in each publication differ and usually just one of these statistical measures is reported, the direct comparison between studies is impaired. To the best of our abilities, an effort was made to be consistent in these comparisons.

3.1.2. Occurrence of BFRs in different regions around the world

Brominated flame retardants are present in dust samples from the indoor environment all over the globe. There are several reports on the levels of BFRs from the five continents; however there is a significant disparity between the numbers of conducted studies. Europe, for instance, gathers the largest number of publications (Figures 3.1.2 and 3.1.3) both for PBDEs (34%) and HBCDDs (67%) followed by North America (particularly United States), Asia, Oceania and Africa (the last one with only two reports on PBDEs).

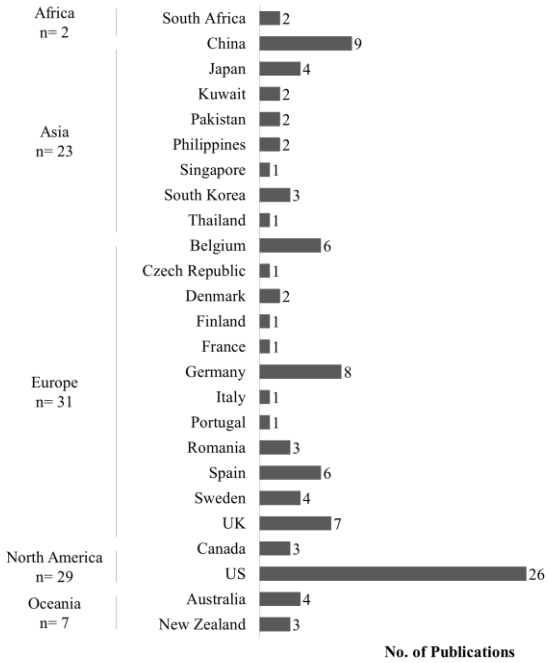

Figure 3.1.2. Number of publications (2003-2013) describing the levels of PBDEs in indoor dust samples across different regions and countries. The number of publications for each region is sometimes lower than the sum of the publications per country because in some studies, samples from different countries (and sometimes even different continents) were included.

While considering the distribution of studies in different countries, instead of different regions, the scenario is different. For PBDEs, for example, the majority of publications resulted from monitoring surveys conducted in the United States (US) with 26 publications, followed by China with 9 publications and then by the following European countries: Germany (n=8), United Kingdom (n=7), Belgium and Spain (6 studies each). In the case of the HBCDDs, the number of publications is less when comparing to PBDEs, and the majority of studies are again from Europe. In terms of countries ranking, the United Kingdom (UK) leads with 7 publications, followed by US and Germany, both with 5 publications.

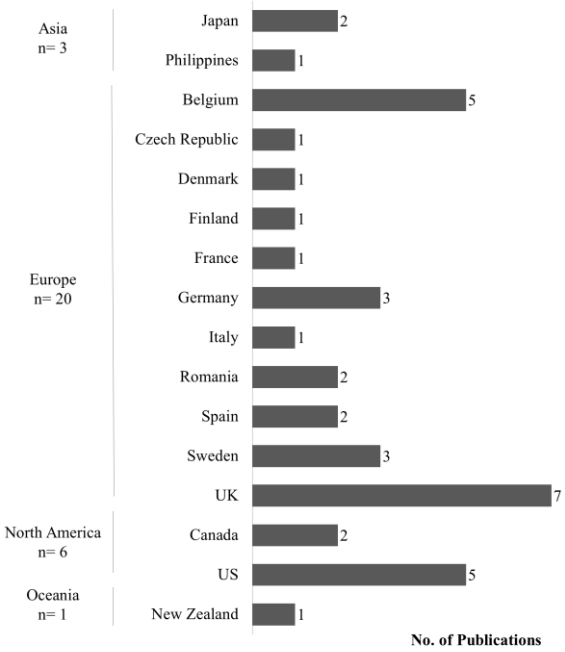

Figure 3.1.3. Number of publications (2003-2013) describing the levels of HBCDDs in indoor dust across different regions and countries. The number of publications for each region is sometimes lower than the sum of the publications per country because in some studies, samples from different countries (and sometimes even different continents) were included.

For the dust collection location, the majority of the published studies (69% for PBDEs and 67% for HBCDDs) focused on indoor dust collected at houses and/or apartments (Figures 3.1.4 and 3.1.5), most probably because in general people spend two-thirds of their indoor time at home (Le Cann et al., 2011). Other collection places included: offices, cars, public places (e.g. schools, universities, hotels and day care centers), several buildings (studies that described together, data from different type of buildings) and garages.

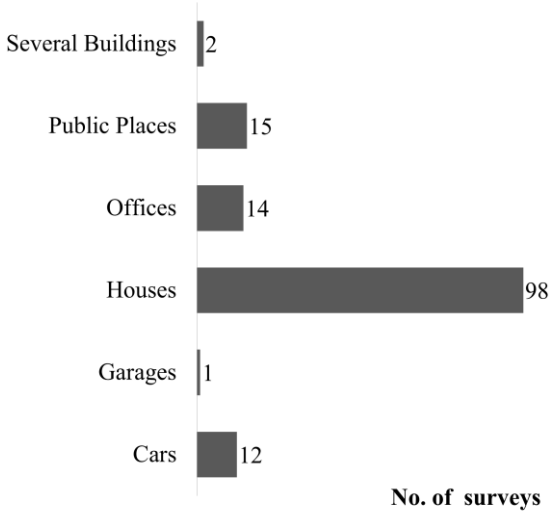

Figure 3.1.4. Number of PBDEs monitoring surveys conducted in cars; garages; houses (including private homes and apartments); offices, public places (including schools, universities, daycare centers and hotels) and several buildings (studies that described together data from different type of buildings).

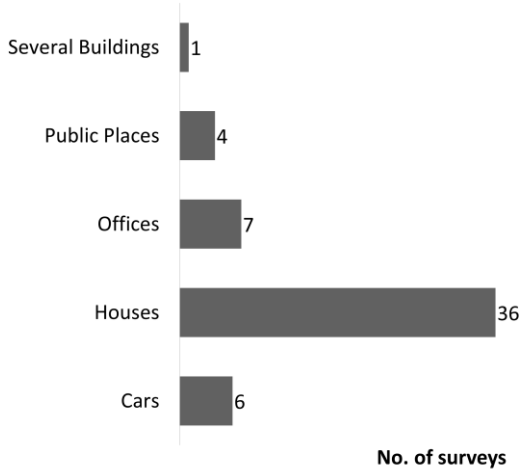

Figure 3.1.5. Number of HBCDDs monitoring surveys conducted in cars; houses (including private homes and apartments); offices, public places (including schools, universities, daycare centers and hotels) and several buildings (studies that described together, data from different type of buildings).

3.1.3. Polybrominated diphenyl ethers

Polybrominated diphenyl ethers (Figure 3.1.6) are, since the 1970s, one of the most widely used brominated flame retardants group incorporated in many consumer goods (Besis et al., 2012).

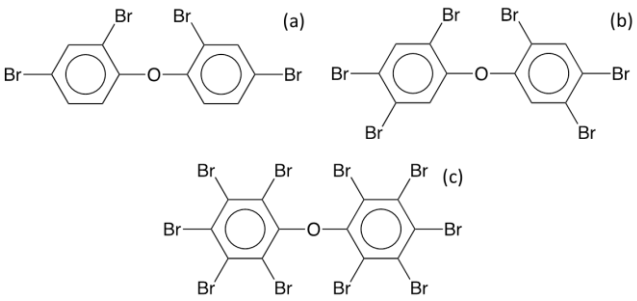

Figure 3.1.6. Structures of (a) BDE-47, (b) BDE-153 and (c) BDE-209.

This class is composed of 209 congeners that differ in the number and position of bromine atoms (US-EPA, 2013). There are three commercial PBDE mixtures manufactured and used in the market, penta-, octa- and deca-BDEs composed of several congeners. Penta-BDE mixture is composed mainly by the congeners 47, 99 and 100 (tetra- and penta-BDEs); octa-BDE mixture consists predominantly of 153, 154, 183 (hexa and hepta-BDEs); deca-BDE mixture composed primarily of BDE-209 congener (Björklund et al., 2012). Although the commercial mixtures have been applied in several items and applications should vary among countries, their incidence is generally associated with specific materials; penta-BDE was primarily used in flexible polyurethane foam in upholstery and furniture, octa-BDE in plastics used in the manufacture of certain electric and electronic equipment's and deca-BDE applied mainly as an additive in electronic devices (US-EPA, 2013). When applied, the mixtures are not chemically bound to the products polymers, and consequently they can migrate/evaporate from the products and are released into the environment (Domingo, et al., 2012). As they have relatively low vapor pressures, PBDEs are classified as semi-volatile organic compounds (SVOCs). PBDEs are able to migrate from consumer products to the indoor environment. Saito et al. (2007), for example, demonstrated that BDE-47 is emitted from the casings of televisions. This team conducted migration tests through solid phase extraction disks placed directly in contact with the product surfaces without air flow to measure the migration rates of the BFRs from the building materials and electrical appliances to indoor air. A further study demonstrated the emission of PBDEs from upholstery textile samples at room temperature, using small stainless steel containers as emission test chambers (Kajiwara et al., 2013). Hazrati and Harrad (2006) reported the occurrence of some variations in the PBDEs levels between

different rooms in the same domestic and office buildings. They reported the decreasing trends in PBDEs concentrations in indoor air from one office after the substitution of a computer produced in 1998 for one produced in 2003. Zhang et al. (2011) also proved that PBDEs sources differ according to location with concentrations in houses lower than those in offices. These authors demonstrated that for areas with higher PBDEs concentrations the main sources were electronic devices. On the other hand, in areas with lower concentrations the main sources were polyurethane foam furniture and carpets.

The extensive occurrence of PBDEs along with its bioaccumulative potential and deleterious effects towards wildlife and humans (see section 3.1.3.2) has become a major global concern, leading to the ban on the manufacture and use of some PBDEs mixtures and consequent international agreements and regulations that were introduced from 2004 onwards. Penta- and octa-BDE formulations were phased out in the European Union and the US in 2004 (Besis et al., 2012; BSEF, 2013) and in 2009 they were listed as persistent organic pollutants (POPs) under the Stockholm Convention (Stockholm Convention, 2013). Deca-BDE was banned in Europe in electric and electronic applications in 2008. In the US, the manufacture was allowed until later, however after December 2013 the Environmental Protection Agency (EPA) banned its production (BSEF, 2013). Although excluded from the market in US and Europe, they are still impregnated in several products, and therefore they are still being released into the environment.

3.1.3.1. PBDEs global patterns in indoor dust

Amongst the large number of PBDEs congeners available, BDE-28, 47, 99, 100, 153, 154, 183 and 209 are considered as main constituents of the technical PBDE mixtures and based on the knowledge about their occurrence in the environment and toxicity, of primary interest by regulatory agencies including the Panel on Contaminants in the Food Chain (CONTAM Panel) from the European Food Safety Authority (EFSA, 2011b). Therefore, in this review, only these eight congeners will be addressed. Figures 3.1.7, 3.1.8 and 3.1.9 illustrate that the median concentrations of these congeners have the same distribution pattern, exhibiting few variations, whatever the samples' origin and collection method used, with BDE-209, 99 and 47 on top followed by BDE-100, 153, 154, 183 and 28. Between these eight investigated congeners BDE-209 was the most common, being

detected at higher concentrations in every region (Figure 3.1.7), regardless of where the sampling took place (Figure 3.1.8). This trend reflects the fact that deca-BDE has been the most common commercial PBDE mixture used on a global scale (Schecter et al., 2005) accounting for 81% and 83% of the global PBDE demand in 1999 and 2001 (cited from EFSA, 2011b). Furthermore its use was voluntarily phased out in the US and Canada only at the end of 2012 and its total application banned only in December 2013, extending their usage when compared with the penta- and octa-BDE mixtures. Penta-BDE was predominantly consumed in North America and octa-BDE was the less used mixture (Schecter et al., 2005; EFSA, 2011b). This usage pattern can be observed when evaluating the results from North America as seen in Figure 3.1.7. Generally, the medians of BDE-47 and BDE-99 concentrations (main constituents of the Penta-BDE mixture) are in the same order of magnitude and are slightly below the BDE-209 medians. The distribution of the median concentrations for each of the eight congeners is similar in all regions; however the levels are higher in North America with the exception of BDE-209 that was detected approximately at the same order of magnitude in all countries, demonstrating its' large and extended use. The highest levels of BDE-209 were registered in dust samples collected in the interior of vehicles, in fact the highest median concentration for BDE-209 (190 000 ng g^{-1}) was detected in car cabins from the UK (Harrad and Abdallah, 2011). This value is even higher than the median value for samples collected inside the car's trunks (2700 ng g^{-1}) also evaluated in the same study. Harrad and Abdallah (2011) justified these high levels with the extensive use of Deca-BDE formulation in UK vehicles, greater than any other formulation. The large difference registered between the concentrations in car cabins and trunks may occur as a result of the wider application of PBDEs in the fabrics and printed circuit boards that are located in the car cabins. In their study, Harrad and Abdallah (2011) also found high levels of BDE-202 (not identified in any commercial formulation) in dust from the car cabin also. The presence of BDE-202 inside the cabins where materials are more exposed to solar radiation than car trunks provides further evidences of the photodebromination of BDE-209. BDE-209 can easily debrominate by photolysis, UV radiation and can biotransformate into lower mass congeners (EFSA, 2011b; Kalachova et al., 2012).

The lowest BDE-209 median concentration in the interior of the cars was also detected in Europe, in the Czech Republic, with 168.5 ng g^{-1} (Kalachova et al., 2012). Even so, this

lowest median level is much higher when compared with the minimum values disclosed by the samples of indoor dust from other sources, suggesting that the exposure to this congener is higher in cars. Despite such high values in car interiors, house dust is the most relevant matrix, as it can be easily inferred by the number of publications dealing with house dust. The highest median concentration for BDE-209 (10,000 ng g^{-1}) was detected in UK by Sjödin et al. (2008). In that study, the researchers compared the concentrations of several PBDE congeners (the ones addressed in this review, excluding BDE-28) in indoor dust between houses from UK, Germany, Australia and US. Dust samples from the US and UK displayed the highest levels of total PBDEs, yet the congener's distribution was similar in all countries with the exception of BDE-209 that was much higher in the UK, whereas, BDE-47 and BDE-99, present in the commercial penta-BDE mixture, were the highest in the US.

Regarding BDE-47 and BDE-99, the highest medians, 3750 ng g^{-1} (Zota et al., 2008) and 5840 ng g^{-1} (Quirós-Alcalá et al., 2011) respectively, were detected in house dust samples from California, reinforcing the fact that penta-BDE usage was largely concentrated in North America (Schecter et al., 2005; Besis et al., 2012) and mostly applied in furniture for sale in California (Zota et al., 2008).

In general terms, and considering dust samples collected from different places (e.g. offices and cars) the BDE-47 and 99 highest median values were all obtained in the US, with the exception of one study performed at Shanghai University. In that survey, levels of BDE-47 and 99 were higher than those reported for public locations in the US. The high levels found at Shanghai University, up to 338 ng g^{-1} for BDE-47 and 503 ng g^{-1} for BDE-99, were detected in one pooled dust sample obtained by brushing air conditioner filters from the university laboratories (Yu et al., 2013). The aim of the study was to compare size-specific concentrations and bioaccessibility of the congeners in dust from air conditioner filters, and thus the authors sieved the sample through several meshes of different sizes and then separated into five size ranges; the mentioned results were obtained from the first particle size range (212–250 μm). In offices, the highest median values were detected by Batterman et al. (2010) in Michigan, with 978 ng g^{-1} (BDE-47) and 1760 ng g^{-1} (BDE-99). In car interiors, the highest levels were also reported in Michigan, with medians 1800 ng g^{-1} (BDE-47) and 2600 ng g^{-1} (BDE-99) (Batterman et al., 2009).

Octa-BDE mixture, predominantly constituted by BDE-183, was generally the minor PBDE product used (Birnbaum et al., 2004). This fact can be easily perceived while considering the maximum levels of BDE-183 compiled here (Figure 3.1.7, 3.1.8 and 3.1.9 and supplementary data in Annex A).

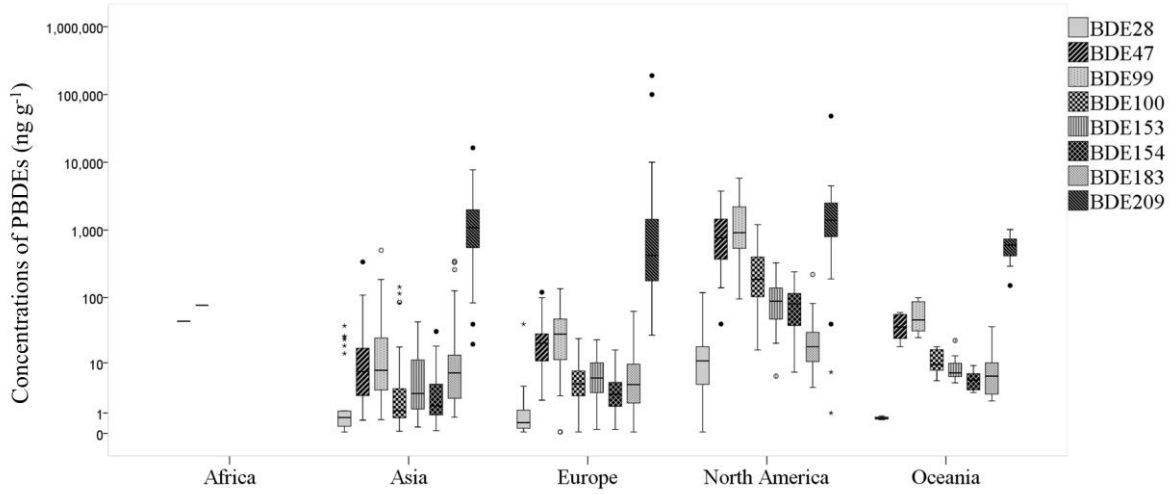

Figure 3.1.7. Concentration range of PBDE congeners in indoor dust samples from Africa, Asia, Europe, North America and Oceania. (Logarithmic scale was used for a better visualization of the distribution).

In fact, BDE-183 maximum concentration values found in indoor dust samples were always below the ones for BDE-47, 99 and 209, with the exception of one study conducted in public places where the maximum concentration of BDE-183 exceeded BDE-47. In this particular case, the BDE-183 maximum value of 354.63 ng g^{-1}[†], was detected in samples collected from day care centers in South Korea (Kim et al., 2011a). This high concentration for BDE-183 is higher than the concentration of BDE-47 detected by Yu et al. (2013) in the laboratories at Shanghai University; however the latter report is the result of one pooled sample while the Korean survey reports the mean concentration of several samples.

[†] this value refers to the mean concentration of several samples collected from day care centers

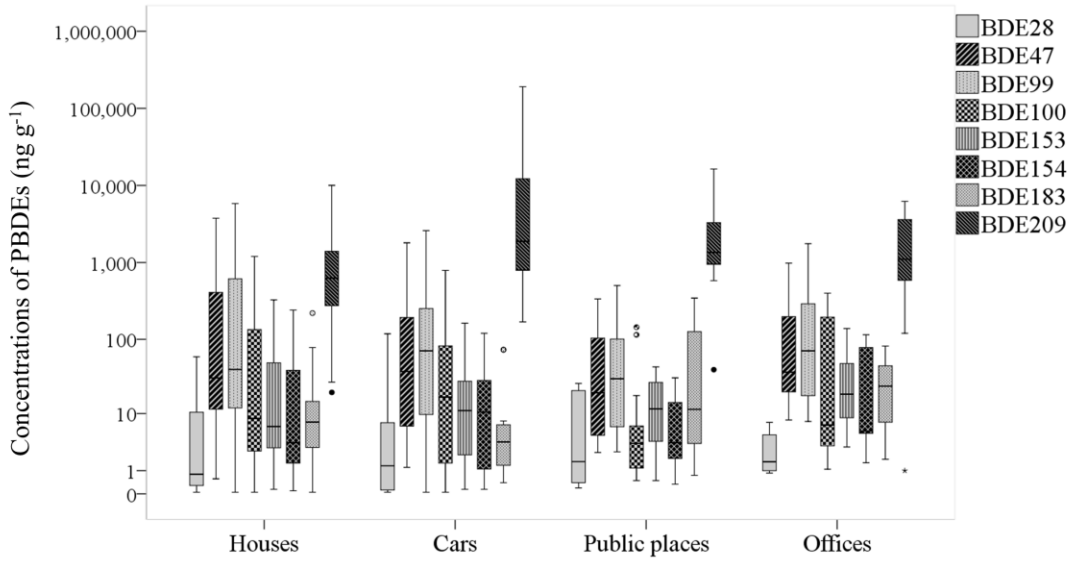

Figure 3.1.8. Concentration range of PBDE congeners in indoor dust samples from different environments; Houses, Cars, Public Places (including schools, universities, daycare centers and hotels) and Offices. (Logarithmic scale was used for a better visualization of the distribution).

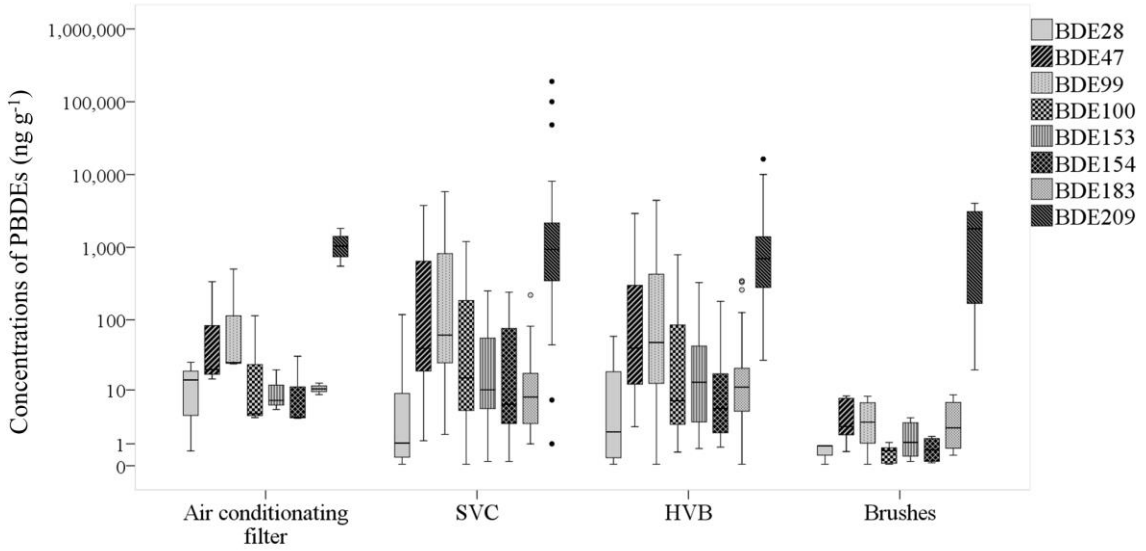

Figure 3.1.9. Concentration range of PBDE congeners in indoor dust samples collected by different methods: SVC- Specific vacuum cleaner or domestic vacuum cleaner with a nylon sock; HVB- Household vacuum cleaner. (Logarithmic scale was used for a better visualization of the distribution).

3.1.3.2. Human Exposure to PBDEs

Humans, as well as wildlife, are constantly exposed to multiple POPs, including PBDEs, due to their common presence not only in many consumer products but also in the

environment. This continuous exposure occurs through different pathways. The main exposure sources are air, dust and diet (Frederiksen et al., 2009), however the indoor exposure, for example indoor dust ingestion and inhalation, could be the major exposure route of PBDEs migrated from the commercial products used in the indoor environment. According to Lorber (2008), concentrations of PBDEs in indoor air and dust are often several orders of magnitude higher than outdoor air and soil, furthermore 82% of the exposure of Americans to BDE congeners is from ingestion of house dust and dermal contact, dust inhalation and ingestion of food and water accounts for the remaining 18% (Lorber, 2008).

In order to understand if dust is, in fact, an important source of PDDEs towards humans, several surveys with matched dust and human samples were conducted over the last years. The goal of such studies was to evaluate possible associations between the levels of PBDEs in dust and those found in human samples including serum, plasma, breast milk and hair (see Table 3.1.1).

In Europe, several studies have been performed and some reported positive associations between PBDEs levels in indoor dust and in human tissues. Karlsson et al. (2007), reported a positive relationship for the sum of PBDEs concentrations in dust and plasma from Sweden. In Denmark, Vorkamp et al. (2011) evaluated PBDEs levels in household dust samples provided by pregnant women and the results disclosed several significant associations between penta-BDE congeners (BDE-99 and BDE-100) levels in these dust samples and the levels in placental tissue collected from the parturients. However, no association was found between BDE-209 levels in dust and in placenta, this despite the fact that BDE 209 was the most abundant congener in both matrices. According to Vorkamp et al. (2011), the obtained results highlight that dust may be an important exposure pathway for penta-BDE congeners. The same research group compared the dust samples with the paired pregnant women plasma and umbilical cord blood plasma samples, and reported positive associations for BDE-28, 47, 100, 209 and the sum of PBDEs in maternal plasma and house dust, as well as the sum of PBDEs in umbilical cord blood plasma and house dust (Frederiksen et al., 2010).

Table 3.1.1. Overview of PBDEs surveys matching indoor dust samples and human samples. ☑ Significant correlation between several PBDEs levels in indoor dust and biological human samples; ☒ No correlation between several PBDEs levels in indoor dust and biological human samples; (*) Altered hormone levels in relation to PBDE exposures estimated as concentrations in house dust.

	Plasma	Serum	Breast milk	Hair	Placenta	Umbilical cord plasma	Hormone levels (men)	Reference
Indoor Dust	☑							Karlsson et al. (2007)
			☑					Wu et al. (2007)
		☒						Zota et al. (2008)
		☒						Fromme et al. (2009)
		☒						Imm et al. (2009)
							☑ (*)	Meeker et al. (2009)
		☒						Roosens et al. (2009b)
			☑					Toms et al. (2009a)
	☑					☑		Frederiksen et al. (2010)
		☑						Johnson et al. (2010)
				☑				Kang et al. (2011)
					☑			Vorkamp et al. (2011)
		☒						Watkins et al. (2011)
				☑				Zheng et al. (2011)
			☑					Björklund et al. (2012)
		☑						Stapleton et al. (2012)
			☑					Coakley et al. (2013)
							☑ (*)	Johnson et al. (2013)
				☑				Tang et al. (2013)

Again in Sweden, BDE-47 concentrations were significantly correlated in matched dust samples from vacuum cleaners and breast milk samples (Björklund et al., 2012). However, not all surveys could identify associations between the concentrations of PBDEs in dust and in human samples. Fromme et al. (2009) and Roosens et al. (2009b), for example, could not identify significant associations in PBDEs concentrations between house dust and blood serum samples from male and female participants.

Considering the surveys performed in the US, although some researchers could not determine significant associations between house dust and blood serum samples (Imm et al., 2009; Watkins et al., 2011), others found associations between the concentrations of some PBDE congeners, especially BDE- 47, 99 and 100, in indoor dust samples and breast

milk and serum samples (Wu et al., 2007; Johnson et al., 2010; Stapleton et al., 2012). Furthermore, PBDEs levels in dust were not only associated with the levels detected in blood samples but were also associated with altered hormone ratios in men, demonstrating that exposure to PBDEs via house dust is responsible for alterations in the normal functioning of male hormonal system. Meeker et al. (2009) and Johnson et al. (2013) analyzed the hormonal levels in men serum from Massachusetts and found evidence of altered levels in relation to concentrations of BDE-47, 99 and 100 in house dust.

In the case of Asia and Oceania, the surveys showed several positive associations between the concentrations of some PBDE congeners (especially BDE-47, 99, 100, 153 and 183) in indoor dust and in breast milk and hair samples (Toms et al., 2009a; Kang et al., 2011; Zheng et al., 2011; Coakley et al., 2013; Tang et al., 2013).

The significant associations observed between PBDE levels in dust and the mentioned biological samples (Table 3.1.1) support the concept that dust is an important source of PBDEs towards humans; nevertheless these associations do not provide information about the main exposure route.

As previously stated, there are several routes such as oral exposure from food, dust, and soil, air inhalation and dermal absorption (Trudel et al., 2011). In order to characterize dust as an important exposure pathway, total PBDEs intake through dust ingestion and inhalation was estimated in several studies. People ingest and inhale considerable amount of dust on a daily basis. This is particularly significant for infants and toddlers because of the repeated hand-to-mouth activities (Mercier et al., 2011), as well as their lower height, that makes them more susceptible to the contact to suspended particles. Thereby, the estimated dust intake for children is higher than the one for adults. There is no consensus on the estimated amounts of inhaled and ingested dust, however the mean dust intake rates, recently updated by the US Environmental Protection Agency, are 30 mg day^{-1} for adults and 60 mg day^{-1} for children (US-EPA, 2011). In order to determine the estimated PBDEs intakes the authors applied different methodologies which impair a straight forward comparison. Concerning the dust intake rates, for example, they may range from 20 mg day^{-1}, average dust ingestion rate (Stapleton et al., 2005) to 215 mg day^{-1}, high dust ingestion rate (D'Hollander et al., 2010) for toddlers and children. For adults, the reported rates range from 4.16 to 100 mg day^{-1} (e.g. Toms et al., 2009b). Since the analyzed congeners vary between surveys, the total PBDEs are also different. Nevertheless and in

general terms, the estimated PBDEs intakes are higher in North America (e.g. Stapleton et al., 2005; Wilford et al., 2005; Harrad et al., 2008; Wei et al., 2009; Shoeib et al., 2012) and then in Asia (e.g. Huang et al., 2010; Kang et al., 2011; Lee et al., 2013) when compared with the rates estimated for Europe (e.g. Fromme et al., 2009; Roosens et al., 2009b; Cunha et al., 2010; Kalachova et al., 2012) and Oceania (e.g. Toms et al., 2009b; Coakley et al., 2013). Such distribution would be expected according to the reported concentrations of the PBDEs in indoor dust.

Once entering the human body, PBDEs elimination may take days, months or even years, depending on the molecular mass of the congener. Thuresson et al. (2006) demonstrated that half-lives of PBDEs in human tend to increase with decreasing bromination of the PBDE congeners, they estimated that apparent half-lives for octa- and nona-BDEs ranged from 37 to 91 and from 18 to 39 days, respectively. For deca-BDE (BDE-209) the human half-life was short when compared to other congeners, 15 days. Thuresson et al. (2006) justified the shorter half-life of BDE-209 by the susceptibility of this congener to undergo reductive dehalogenation and substitution reactions. Short half-life of BDE-209 can also be justified by the fact that its absorption after ingestion is limited and so it is rapidly excreted through feces, with little accumulation in tissues, besides it does not easily penetrate the cell membranes (Costa and Giordano, 2011). Trudel et al. (2011) reported median elimination half-lives of 1.4; 0.77; 1.8 and 7.4 years for BDE-47, 99, 100 and 153 respectively and 7 days for BDE-209. In another survey, Wong et al. (2013), estimated 0.37; 8.2; 2 and 3.5 years also for BDE-47, 99, 100 and 153 respectively.

These compounds have the potential to cause deleterious effects on human health and therefore several epidemiological, *in vivo* and *in vitro* studies have been conducted with the aim to confirm their endocrine disrupting capacity (e.g. disturbance of thyroid hormone homeostasis) and their effects in the neurodevelopment and reproductive system (Birnbaum and Staskal, 2004; Costa et al., 2008). Lilienthal et al. (2006) performed an experiment with rats in which the results support the hypothesis that PBDEs have endocrine disrupting potential interfering with the sexual development and sexually dimorphic behavior. Main et al. (2007) further described an association between congenital cryptorchidism in newborn boys and the penta-BDE concentrations in the mother's breast milk. Two distinct surveys, performed in Europe (Roze et al., 2009) and US (Herbstman et

al., 2010), reported neurodevelopmental and neurobehavioral effects in children associated with PBDEs exposure.

Despite the already available information on the levels, behaviour and deleterious effects of the PBDEs, it is important to continue monitoring surveys and epidemiological studies so that the PBDE congeners toxicity in humans is confirmed.

3.1.4. Hexabromocyclododecanes

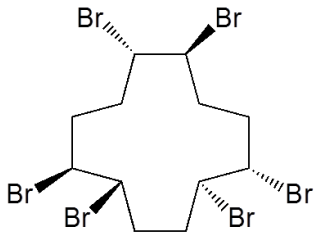

Figure 3.1.10. Structure of gamma-HBCDD.

Hexabromocyclododecanes (HBCDDs) (Figure 3.1.10) are cycloaliphatic brominated flame retardants produced globally and used primarily as additive in extruded and expanded polystyrene foam materials in the building industry and in textile coating, with minor applications in electrical and electronic equipment (Marvin et al., 2011). HBCDDs are produced since the 1960s, however the application in insulation boards started only in the 1980s (EFSA, 2011a). The introduction of PBDEs regulations led to an increase of HBCDDs demand (Covaci et al., 2012). In 2001, the total production volume of HBCDDs was 16,700 tons of which 9,500 tons were consumed in Europe (Abdallah et al., 2008b). In 2011, the estimated annual production was approximately 28,000 tons (9,000 to 15,000 tons in China; 13,426 tons in Europe and US). HBCDDs are produced in China, Europe, Japan and US, however they are mainly used in Europe and China (Stockholm Convention, 2011). Annual market demand in Japan was 2800 tons in 2011 (Ministry of the Environment, 2014).

The commercial HBCDD product is dominated by three diastereoisomers: alpha (α-), beta (β-) and gamma (γ-) HBCDDs (EFSA, 2011a). The relative contribution of these three isomers in the technical HBCDDs depends on the manufacturer; generally γ-HBCDD accounts for 72 to 90 % of the total and α- and β-HBCDD contribute with 9 to 13 % and with <0.5 to 12 %, respectively (EFSA, 2011a).

HBCDDs are known to be hydrophobic, to have low vapor pressure (Abdallah et al., 2008b), to be highly lipophilic and to have a high affinity to particulate matter (Stockholm Convention, 2011). Like PBDEs, they are persistent, bioconcentrate and biomagnify at higher trophic levels and are present at high concentrations in top predators such as marine mammals and birds of prey (Covaci et al., 2006). Due to their widespread distribution in the environment, including in remote locations such as the Arctic (Marvin et al., 2011), and their persistency, a global concern on HBCDDs has emerged in the last few years. In 2008, Norway prepared a draft dossier proposing HBCDD as a POP candidate, and finally in 2010, the POPs Review Committee concluded that it gathered the criteria (long-range environmental transport, significant adverse human health and environmental effects) to adopt the Risk Profile (Stockholm Convention, 2011; BSEF, 2013). In May 2013, it was listed as a POP by the Conference of the Parties of the UNEP (United Nations Environment Programme) Stockholm Convention on Persistent Organic Pollutants (BSEF, 2013). In Europe, HBCDD was registered under REACH (Registration, Evaluation, Authorization and Registration of Chemicals) in October 2010 and the European ban is scheduled to 21 August 2015 unless authorized for specific uses (BSEF, 2013). Regardless of such facts, the market demand for HBCDDs has been growing, as well as, their distribution in the environment, thus rendering HBCDDs monitoring crucial.

3.1.4.1. HBCDDs global patterns in indoor dust

In contrast to the PBDEs, there is limited information about the concentrations of HBCDDs in indoor dust, the number of publication is considerably lower (38 for HBCDDs and 103 for PBDEs) and the studies were mainly from Europe (UK and Belgium) (Figure 3.1.3).

Generally, in most of the performed surveys HBCDDs concentrations are reported as the sum of the three HBCDD isomers, and only few reported the concentration levels for each isomer individually. The highest levels were reported in Europe (Figure 3.1.11) followed by Asia. Such distribution would be expected since, as previously mentioned, the largest part of the technical HBCDDs is used in Europe. In each of the four regions represented in Figure 3.1.11, it is possible to notice that γ-HBCDD has the highest median concentrations and follows a similar distribution to total HBCDD, whereas α-HBCDD levels are slightly

lower, followed by β-HBCDD. This pattern (γ-HBCDD>α-HBCDD>β-HBCDD) reflects the HBCDDs commercial mixture composition. The same distribution can be perceived considering the dust samples origin (Figure 3.1.12). The highest HBCDDs concentrations were detected in car dust samples (Figure 3.1.12). The highest median concentration for total HBCDDs in car dust (14,262.5 ng g^{-1}‡) was detected in the UK by Abdallah and Harrad (2009), as well as the highest median concentration for the γ-HBCDD isomer (9651 ng g^{-1}). Considering α-HBCDD and β-HBCDD, maximum median concentrations, 3000 and 1100 ng g^{-1} respectively, were detected by the same researchers in car cabins, also from the UK (Harrad et al., 2011). As previously mentioned (section 3.1.3.1), this study compared the concentrations between the car cabins and trunks and the median concentrations were higher in the cabin. The authors suggested that such results might be a consequence of a possible photolytic or thermal isomerization from γ-HBCDD to α-HBCDD.

In house dust, the highest concentration for total HBCDDs (6570 ng g^{-1}§) was found in Japan by Takigami et al. (2009). Such high level refers to the mean concentration of the total HBCDDs in two recently built wooden houses in Hokkaido (Northern Japan). In individual terms, concentration of HBCDDs was extremely high at one of the houses (13,000 ng g^{-1}) being two orders of magnitude higher than the one found for PBDEs (Takigami et al., 2009). The maximum concentrations of HBCDDs in house dust, in terms of the three isomers individually, were detected by Harrad et al. (2009) in three different houses. In this study the authors analysed dust samples from five different rooms at each house and the mean concentrations§ for each isomer, were 1881 ng g^{-1} (α-HBCDD), 570 ng g^{-1} (β-HBCDD) and 1702 ng g^{-1} (γ-HBCDD).

In what concerns to offices and public places the highest total and individual isomer concentrations were detected again in the UK by Harrad et al. (2009) and Harrad et al. (2010b), respectively. A total median HBCDDs concentration of 4100 ng g^{-1} was reported in dust samples from classrooms in child day care centers and primary schools in the West Midlands of the UK.

‡ the median values were not reported, we calculated them from the reported concentrations

§ this value refers to the mean concentration (we calculated the mean from the reported concentrations)

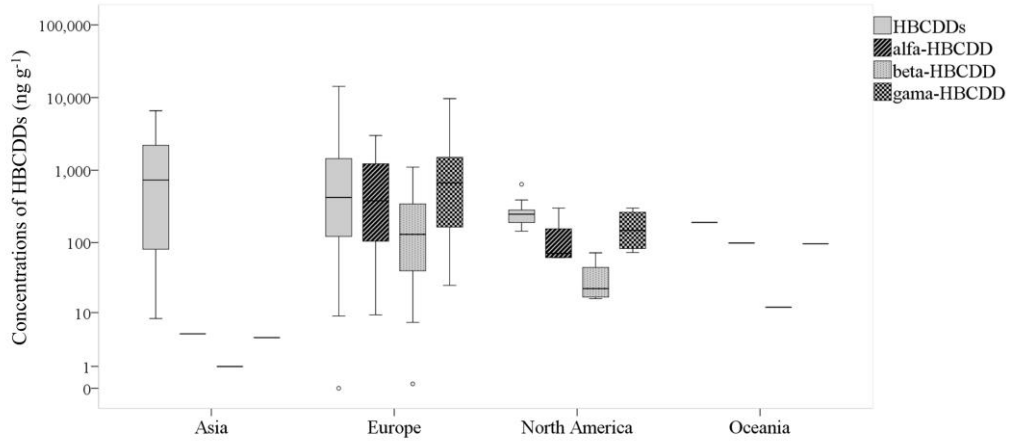

Figure 3.1.11. Concentration range of total HBCDDs and HBCDD isomers in indoor dust samples from Africa, Asia, Europe, North America and Oceania. (Logarithmic scale was used for a better visualization of the distribution).

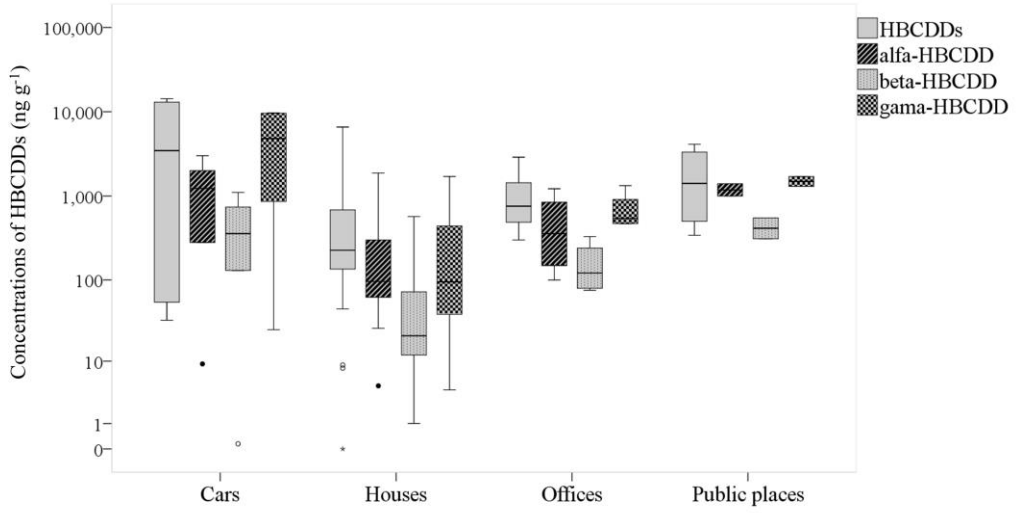

Figure 3.1.12. Concentration range of total HBCDDs and HBCDD isomers in indoor dust samples from different environments; Houses, Cars, Offices, Public Places (including schools, universities, daycare centers and hotels) and Offices. (Logarithmic scale was used for a better visualization of the distribution).

3.1.4.2. Human Exposure to HBCDDs

Similarly to what happens for other FRs, human exposure to HBCDDs occurs via multiple sources, including diet, indoor air and dust (Covaci et al., 2006). Due to the high HBCDDs concentrations detected in indoor dust samples (see section 3.1.4.1 and supplementary

material) and considering the time people spend at home, house dust seems to be an important source of HBCDDs (Harrad et al., 2010a).

Several studies have been performed in order to quantify the levels of HBCDDs in human samples. HBCDDs have already been detected in serum (e.g. Roosens et al., 2009a), adipose tissue (e.g. Antignac et al., 2008; Isobe et al., 2009) and breast milk (e.g. Eljarrat et al.,2009). Breast milk is a particularly suitable matrix as it is considered to mirror the market consumption of HBCDDs (Marvin et al., 2011; Stockholm Convention, 2011). Although there are several publications reporting the levels in human tissues, few surveys evaluated the association between the levels in dust and biological samples, to confirm the fact that indoor dust works as an important HBCDDs source to humans.

Roosens et al. (2009a) quantified the levels of the HBCDDs in duplicate diet samples, provided by Belgium students residing in university housing, in dust samples collected at the student's dorms and in serum samples from the participants. The authors found a positive association between the HBCDDs levels in serum and the exposure *via* dust but not with dietary ingestion. In the US, Johnson et al. (2013) found evidences of altered hormonal levels in men blood serum related to the HBCDDs concentrations in house dust similarly of what was reported for PBDEs (see section 3.1.3.2). Even if there is little information, these positive associations between HBCDDs levels in indoor dust and in biological samples, demonstrate that dust is an important source regarding the human exposure to this compound.

For the HBCDDs, the human daily intake is also estimated by several studies considering the different dust intake by adults and toddlers. As in PBDEs surveys, the assumed mean and high dust ingestion rates vary between studies. Considering toddlers, the dust intake rates range from the mean dust ingestion rate of 50 ng g^{-1} (e.g. Abdallah et al., 2008b) to the high dust ingestion rate of 215 ng g^{-1} (D'Hollander et al., 2010). For adults, the ingestion rates range from 4.16 ng g^{-1} (Takigami et al., 2009) to 50 ng g^{-1} (e.g. Kalachova et al., 2012). As already mentioned, due to the reduced number of surveys conducted in North America when compared to Europe, it is difficult to compare the estimated HBCDDs intakes *via* dust ingestion between regions. Within the discussed studies, Abdallah et al. (2008a, b) found the highest estimated HBCDDs human intakes in Europe for both adults and children. In addition, Abdallah et al. (2008b) disclosed higher intakes in the UK when compared with the results from Canada and US. On a global basis, the

highest estimated intake was reported in Japan, for the wooden house where the highest HBCDDs concentrations were reported (see section 3.1.4.1) with intake values between 55-1300 ng g^{-1} for adults and 720-2600 ng g^{-1} for children.

HBCDDs elimination from human body is estimated to take several days. Geyer et al. (2004) estimated a human elimination half-life of 64 days, ranging from 23 to 219 days and they justify this variability with the differences in exposure, total body fat content and biological differences that can affect the intake, elimination and bioaccumulation. Based on this study, Abdallah and Harrad (2011) calculated different half-life values for each diastereomer, 165 days for α-HBCDD (representing 75% of the maximum half-life of 219 days for the HBCDDs mixture), and a half-life of 55 days for the β- and γ-isomers (25% of 219 days).

As well as PBDEs, HBCDDs may also cause deleterious health effects in humans. HBCDDs can interfere with the thyroid function, the reproductive system and it is associated with neurobehavioral alterations (EFSA, 2011a). Several *in vivo* and *in vitro* studies confirmed the potential of HBCDDs to disrupt the thyroid homeostasis. van der Ven et al. (2006), for example, performed a 28-day oral dose toxicity assay in rats and reported dose-related effects on the thyroid hormone. Eriksson et al. (2006) showed through a developmental neurotoxicity study in mice, that HBCDDs induced effects in behaviour, learning and memory function. These studies conducted in rodents report critical effects in the normal organism function and may explain what can occur in humans. Therefore, as in the case of other flame retardants, it is mandatory to investigate the possible associations between HBCDDs exposure and deleterious health outcomes.

3.1.5. Conclusions

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs) are brominated flame retardant extensively used over the past few decades and are ubiquitous in the indoor environment. Levels of these chemicals in indoor dust are generally high as dust acts as a concentrator and repository of these substances. Furthermore, dust is considered as one of the main exposure pathways for humans. In this review we compiled the levels of these FRs in dust samples from different origins (houses, offices, public places and cars) on a worldwide basis. Overall, the number of studies dealing with PBDEs

levels in indoor dust is much higher than the ones performed for HBCDDs. Generally, concentrations of PBDEs (especially BDE-47 and BDE-99) are higher in the North America, reflecting its extensive application in consumer products. Regarding the HBCDDs, levels are particularly higher in Europe as their production and usage is mainly concentrated in European countries. However, due to the limited number of studies performed outside Europe it is difficult to truly understand the HBCDDs global distribution. The concern about the effects of these flame retardants is global. Despite the fact that there is already a good amount of information on the concentrations of these contaminants in dust samples, on their behaviour and possible deleterious effects in humans, there are some difficulties when trying to compare results between the published studies. Such difficulties are associated with different sampling strategies, different target chemicals analysed and different ways of expressing the results and calculating ingestions rates. The use of different sampling methods, including household vacuum cleaner, specific vacuum cleaner (provided by the researchers), domestic vacuum cleaner with a nylon sock, or brushes and wipes for example, generates different dust samples that are difficult to compare. Furthermore, the analytical methods used to quantify PBDEs and HBCDDs may also differ between laboratories, and the target chemicals analyzed are not always the same. Although BDE-28, 47, 99, 100, 153, 154, 183 and 209 are considered, by EFSA, the congeners of primary interest, the congeners selected for analysis vary considerably between studies, thus the resulting sum of PBDEs also differs, rendering the comparison between studies impossible. In data analysis, the fact that some studies report the results in medians and others in mean or geometric mean also renders the direct comparison difficult, besides temporal comparisons are also difficult to perform as some studies do not refer the sample collection dates. Ideally, the monitoring surveys should follow some general guidelines in order to allow more straight forward comparisons between studies and therefore to better access information on the effects of human exposure to these contaminants from different regions worldwide. Finally, such surveys will allow evaluating the effectiveness of the legislation implemented over the years.

Acknowledgements

This work was financially supported by Portuguese Foundation for Science and Technology FCT trough program COMPETE (Projects PEst-C/MAR/LA0017/2011; PEst-C/SAU/UI0709/2011 and

grants SFRH/BD/78168/2011; SFRH/BPD/65884/2009). Further support was provided by Grants-in-Aid for Scientific Research (S: 26220103, A: 25257403, A: 25241013, B: 25281008) of the Japanese Ministry of Education, Science, Sports, Culture and Technology (MEXT) and Japan Society for the Promotion of Science (JSPS).

The authors would also like to thank the assistance of Tiago Maricoto, MD in data analysis using IBM SPSS Statistics 20 software; and Jorge Dias, MDes for kindly providing the illustration for Figure 3.1.1. The authors are also thankful to Dr. A. Subramanian (CMES, Ehime University) for the critical reading of the manuscript.

Supplementary Data

Supplementary data to this subchapter can be found in Annex A.

References

Abdallah, M.A.-E., Harrad, S., 2011. Tetrabromobisphenol-A, hexabromocyclododecane and its degradation products in UK human milk: Relationship to external exposure. Environ. Int. 37, 443-448.

Abdallah, M.A.-E., Harrad, S., Covaci, A., 2008a. Hexabromocyclododecanes and tetrabromobisphenol-A in indoor air and dust in Birmingham, UK: Implications for human exposure. Environ. Sci. Technol. 42, 6855-6861.

Abdallah, M.A.E., Harrad, S., 2009. Personal exposure to HBCDs and its degradation products via ingestion of indoor dust. Environ. Int. 35, 870-876.

Abdallah, M.A.E., Harrad, S., Ibarra, C., Diamond, M., Melymuk, L., Robson, M., Covaci, A., 2008b. Hexabromocyclododecanes in indoor dust from Canada, the United Kingdom, and the United States. Environ. Sci. Technol. 42, 459-464.

Allen, J.G., McClean, M.D., Stapleton, H.M., Webster, T.F., 2008. Critical factors in assessing exposure to PBDEs via house dust. Environ. Int. 34, 1085-1091.

Antignac, J.P., Cariou, R., Maume, D., Marchand, P., Monteau, F., Zalko, D., Berrebi, A., Cravedi, J.P., Andre, F., Le Bizec, B., 2008. Exposure assessment of fetus and newborn to brominated flame retardants in France: preliminary data. Mol. Nutr. Food Res. 52, 258-265.

Batterman, S., Godwin, C., Chernyak, S., Jia, C., Charles, S., 2010. Brominated flame retardants in offices in Michigan, U.S.A. Environ. Int. 36, 548-556.

Batterman, S.A., Chernyak, S., Jia, C., Godwin, C., Charles, S., 2009. Concentrations and emissions of polybrominated diphenyl ethers from U.S. houses and garages. Environ. Sci. Technol. 43, 2693-2700.

Besis, A., Samara, C., 2012. Polybrominated diphenyl ethers (PBDEs) in the indoor and outdoor environments - A review on occurrence and human exposure. Environ. Pollut. 169, 217-229.

Birnbaum, L.S., Staskal, D.F., 2004. Brominated flame retardants: Cause for concern? Environ. Health Persp. 112, 9-17.

Björklund, J.A., Sellström, U., de Wit, C.A., Aune, M., Lignell, S., Darnerud, P.O., 2012. Comparisons of polybrominated diphenyl ether and hexabromocyclododecane concentrations in dust collected with two sampling methods and matched breast milk samples. Indoor Air 22, 279-288.

BSEF, 2013. Bromine Science and Environmental Forum – Regulation. http://www.bsef.com/regulation. (accessed December, 2013).

Butt, C.M., Diamond, M.L., Truong, J., Ikonomou, M.G., Ter Schure, A.F.H., 2004. Spatial Distribution of Polybrominated Diphenyl Ethers in Southern Ontario As Measured in Indoor and Outdoor Window Organic Films. Environ. Sci. Technol. 38, 724-731.

Ceresana, 2011. Market Study: Flame Retardants (2nd Edition).

Ceresana, 2014. Market Study: Flame Retardants (3rd Edition). pp. 380.

Coakley, J.D., Harrad, S.J., Goosey, E., Ali, N., Dirtu, A.C., Van den Eede, N., Covaci, A., Douwes, J., Mannetje, A., 2013. Concentrations of polybrominated diphenyl ethers in matched samples of indoor dust and breast milk in New Zealand. Environ. Int. 59, 255-261.

Costa, L.G., Giordano, G., 2011. Is decabromodiphenyl ether (BDE-209) a developmental neurotoxicant? Neurotoxicology 32, 9-24.

Costa, L.G., Giordano, G., Tagliaferri, S., Caglieri, A., Mutti, A., 2008. Polybrominated diphenyl ether (PBDE) flame retardants: Environmental contamination, human body burden and potential adverse health effects. Acta Biomed. Ateneo Parmense 79, 172-183.

Covaci, A., Geens, T., Roosens, L., Ali, N., Van den Eede, N., Ionas, A., Malarvannan, G., Dirtu, A., 2012. Human Exposure and Health Risks to Emerging Organic Contaminants. The Handbook of Environmental Chemistry. Springer Berlin / Heidelberg, pp. 1-63.

Covaci, A., Gerecke, A.C., Law, R.J., Voorspoels, S., Kohler, M., Heeb, N.V., Leslie, H., Allchin, C.R., de Boer, J., 2006. Hexabromocyclododecanes (HBCDs) in the Environment and Humans: A Review. Environ. Sci. Technol. 40, 3679-3688.

Covaci, A., Harrad, S., Abdallah, M.A.E., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532-556.

Covaci, A., Voorspoels, S., Abdallah, M.A.E., Geens, T., Harrad, S., Law, R.J., 2009. Analytical and environmental aspects of the flame retardant tetrabromobisphenol-A and its derivatives. J. Chromatogr. A 1216, 346-363.

Cunha, S.C., Kalachova, K., Pulkrabova, J., Fernandes, J.O., Oliveira, M.B.P.P., Alves, A., Hajslova, J., 2010. Polybrominated diphenyl ethers (PBDEs) contents in house and car dust of Portugal by pressurized liquid extraction (PLE) and gas chromatography-mass spectrometry (GC-MS). Chemosphere 78, 1263-1271.

D'Hollander, W., Roosens, L., Covaci, A., Cornelis, C., Reynders, H., Campenhout, K.V., Voogt, P.d., Bervoets, L., 2010. Brominated flame retardants and perfluorinated compounds in indoor dust from homes and offices in Flanders, Belgium. Chemosphere 81, 478-487.

Dodson, R.E., Perovich, L.J., Covaci, A., Van Den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., Rudel, R.A., 2012. After the PBDE phase-out: A broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol. 46, 13056-13066.

Domingo, J.L., 2012. Polybrominated diphenyl ethers in food and human dietary exposure: A review of the recent scientific literature. Food Chem. Toxicol. 50, 238-249.

EFSA, 2011a. Scientific Opinion on Hexabromocyclododecanes (HBCDDs) in Food. EFSA J. 118.

EFSA, 2011b. Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA J. 274.

Eljarrat, E., Guerra, P., Martínez, E., Farré, M., Alvarez, J.G., López-Teijón, M., Barceló, D., 2009. Hexabromocyclododecane in Human Breast Milk: Levels and Enantiomeric Patterns. Environ. Sci. Technol. 43, 1940-1946.

Eriksson, P., Fisher, C., Wallin, M., Jakobsson E., Fredriksson A., 2006. Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ. Toxicol. Pharmacol. 21, 317-322.

Frederiksen, M., Thomsen, C., Frøshaug, M., Vorkamp, K., Thomsen, M., Becher, G., Knudsen, L.E., 2010. Polybrominated diphenyl ethers in paired samples of maternal and umbilical cord blood plasma and associations with house dust in a Danish cohort. Int. J. Hyg. Environ. Health 213, 233-242.

Frederiksen, M., Vorkamp, K., Thomsen, M., Knudsen, L.E., 2009. Human internal and external exposure to PBDEs - A review of levels and sources. Int. J. Hyg. Environ. Health 212, 109-134.

Fromme, H., Körner, W., Shahin, N., Wanner, A., Albrecht, M., Boehmer, S., Parlar, H., Mayer, R., Liebl, B., Bolte, G., 2009. Human exposure to polybrominated diphenyl ethers (PBDE), as evidenced by data from a duplicate diet study, indoor air, house dust, and biomonitoring in Germany. Environ. Int. 35, 1125-1135.

Geyer, H.J., Schramm, K.-W., Darnerud, P.O., Aune, M., Feicht, E.A., Fried, K.W., Henkelmann, B., Lenoir, D., Schmid, P., McDonald, T.A., 2004. Terminal elimination half-lives of the

brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. Dioxin. Organohalogen Compounds, pp. 3867-3872.

Harrad, S., Abdallah, M.A.-E., 2011. Brominated flame retardants in dust from UK cars - Within-vehicle spatial variability, evidence for degradation and exposure implications. Chemosphere 82, 1240-1245.

Harrad, S., Abdallah, M.A.E., Covaci, A., 2009. Causes of variability in concentrations and diastereomer patterns of hexabromocyclododecanes in indoor dust. Environ. Int. 35, 573-579.

Harrad, S., De Wit, C.A., Abdallah, M.A.E., Bergh, C., Björklund, J.A., Covaci, A., Darnerud, P.O., De Boer, J., Diamond, M., Huber, S., Leonards, P., Mandalakis, M., Östman, C., Haug, L.S., Thomsen, C., Webster, T.F., 2010a. Indoor contamination with hexabromocyclododecanes, polybrominated diphenyl ethers, and perfluoroalkyl compounds: An important exposure pathway for people? Environ. Sci. Technol. 44, 3221-3231.

Harrad, S., Goosey, E., Desborough, J., Abdallah, M.A.E., Roosens, L., Covaci, A., 2010b. Dust from U.K. primary school classrooms and daycare centers: The significance of dust as a pathway of exposure of young U.K. children to brominated flame retardants and polychlorinated biphenyls. Environ. Sci. Technol. 44, 4198-4202.

Harrad, S., Ibarra, C., Diamond, M., Melymuk, L., Robson, M., Douwes, J., Roosens, L., Dirtu, A.C., Covaci, A., 2008. Polybrominated diphenyl ethers in domestic indoor dust from Canada, New Zealand, United Kingdom and United States. Environ. Int. 34, 232-238.

Hazrati, S., Harrad, S., 2006. Causes of variability in concentrations of polychlorinated biphenyls and polybrominated diphenyl ethers in indoor air. Environ. Sci. Technol. 40, 7584-7589.

Herbstman, J.B., Sjödin, A., Kurzon, M., Lederman, S.A., Jones, R.S., Rauh, V., Needham, L.L., Tang, D., Niedzwiecki, M., Wang, R.Y., Perera, F., 2010. Prenatal Exposure to PBDEs and Neurodevelopment. Environ. Health Perspect. 118, 712-719.

Huang, Y.M., Chen, L.G., Xu, Z.C., Peng, X.C., Wen, L.J., Zhang, S.K., Zeng, M., Ye, Z.X., 2010. Preliminary study of PBDE levels in house dust and human exposure to PBDEs via dust ingestion. Huanjing Kexue/Environ. Sci. 31, 168-172.

Hwang, H.-M., Park, E.-K., Young, T.M., Hammock, B.D., 2008. Occurrence of endocrine-disrupting chemicals in indoor dust. Sci. Total. Environ. 404, 26-35.

Imm, P., Knobeloch, L., Buelow, C., Anderson, H.A., 2009. Household exposures to polybrominated diphenyl ethers (PBDEs) in a Wisconsin Cohort. Environ. Health Persp. 117, 1890-1895.

Isobe, T., Oda, H., Takayanagi, N., Kunisue, T., Komori, H., Arita, N., Ueda, N., Nose, M., Yamada, T., Takahashi, S., Tanabe, S., 2009. Hexabromocyclododecanes in human adipose tissue from Japan. Environ. Chem. 6, 328-333.

Johnson, P.I., Stapleton, H.M., Mukherjee, B., Hauser, R., Meeker, J.D., 2013. Associations between brominated flame retardants in house dust and hormone levels in men. Sci. Total. Environ. 445-446, 177-184.

Johnson, P.I., Stapleton, H.M., Sjodin, A., Meeker, J.D., 2010. Relationships between Polybrominated Diphenyl Ether Concentrations in House Dust and Serum. Environ. Sci. Technol. 44, 5627-5632.

Kajiwara, N., Takigami, H., 2013. Emission behavior of hexabromocyclododecanes and polybrominated diphenyl ethers from flame-retardant-treated textiles. Environ. Sci. Process. Impacts 15, 1957-1963.

Kalachova, K., Hradkova, P., Lankova, D., Hajslova, J., Pulkrabova, J., 2012. Occurrence of brominated flame retardants in household and car dust from the Czech Republic. Sci. Total. Environ. 441, 182-193.

Kang, Y., Wang, H.S., Cheung, K.C., Wong, M.H., 2011. Polybrominated diphenyl ethers (PBDEs) in indoor dust and human hair. Atmos. Environ. 45, 2386-2393.

Karlsson, M., Julander, A., van Bavel, B., Hardell, L., 2007. Levels of brominated flame retardants in blood in relation to levels in household air and dust. Environ. Int. 33, 62-69.

Kim, H.H., Yang, J.Y., Jang, Y.S., Lee, Y.J., Lee, C.S., Shin, D.C., Lim, Y.W., 2011a. Exposure assessment and health risk of Polybrominated Diphenyl Ether (PBDE) flame retardants in indoor environments of children's facilities in Korea. Asian J. Atmos. Environ. 5, 247-262.

Kim, J.-W., Isobe, T., Chang, K.-H., Amano, A., Maneja, R.H., Zamora, P.B., Siringan, F.P., Tanabe, S., 2011b. Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines. Environ. Pollut. 159, 3653-3659.

Le Cann, P., Bonvallot, N., Glorennec, P., Deguen, S., Goeury, C., Le Bot, B., 2011. Indoor environment and children's health: Recent developments in chemical, biological, physical and social aspects. Int. J. Hyg. Environ. Health 215, 1-18.

Lee, S., Kannan, K., Moon, H.B., 2013. Assessment of exposure to polybrominated diphenyl ethers (PBDEs) via seafood consumption and dust ingestion in Korea. Sci. Total. Environ. 443, 24-30.

Lilienthal, H., Hack, A., Roth-H,,rer, A., Grande, S.W., Talsness, C.E., 2006. Effects of Developmental Exposure to 2,2',4,4',5-Pentabromodiphenyl Ether (PBDE-99) on Sex Steroids, Sexual Development, and Sexually Dimorphic Behavior in Rats Environ. Health Persp. 114, 194-201.

Lioy, P.J., Freeman, N.C.G., Millette, J.R., 2002. Dust: A metric for use in residential and building exposure assessment and source characterization. Environ. Health Persp. 110, 969-983.

Lorber, M., 2008. Exposure of Americans to polybrominated diphenyl ethers. J. Expos. Sci. Environ. Epidemiol. 18, 2-19.

Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J., Vartiainen, T., Skakkebæk, N.E., Toppari, J., 2007. Flame Retardants in Placenta and Breast Milk and Cryptorchidism in Newborn Boys Environ. Health Persp. 115, 1519–1526

Marklund, A., Andersson, B., Haglund, P., 2003. Screening of organophosphorus compounds and their distribution in various indoor environments. Chemosphere 53, 1137–1146.

Marvin, C.H., Tomy, G.T., Armitage, J.M., Arnot, J.A., McCarty, L., Covaci, A., Palace, V., 2011. Hexabromocyclododecane: Current Understanding of Chemistry, Environmental Fate and Toxicology and Implications for Global Management. Environ. Sci. Technol. 45, 8613-8623.

Meeker, J.D., 2012. Exposure to environmental endocrine disruptors and child development. Arch. Pediatr. Adolesc. Med. 166, 952-958.

Meeker, J.D., Johnson, P.I., Camann, D., Hauser, R., 2009. Polybrominated diphenyl ether (PBDE) concentrations in house dust are related to hormone levels in men. Sci. Total. Environ. 407, 3425-3429.

Meeker, J.D., Stapleton, H.M., 2010. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ. Health Persp. 118, 318-323.

Mercier, F., Glorennec, P., Thomas, O., Bot, B.L., 2011. Organic contamination of settled house dust, a review for exposure assessment purposes. Environ. Sci. Technol. 45, 6716-6727.

Ministry of the Environment, 2013. http://www.env.go.jp/chemi/dioxin/chosa/result_h24.pdf. (accessed May, 2014).

Papachlimitzou, A., Barber, J.L., Losada, S., Bersuder, P., Law, R.J., 2012. A review of the analysis of novel brominated flame retardants. J. Chromatogr. A 1219, 15-28.

Quirós-Alcalá, L., Bradman, A., Nishioka, M., Harnly, M.E., Hubbard, A., McKone, T.E., Eskenazi, B., 2011. Concentrations and loadings of polybrominated diphenyl ethers in dust from low-income households in California. Environ. Int. 37, 592-596.

Roosens, L., Abdallah, M.A.E., Harrad, S., Neels, H., Covaci, A., 2009a. Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: Preliminary results. Environ. Health Persp. 117, 1707-1712.

Roosens, L., Abdallah, M.A.E., Harrad, S., Neels, H., Covaci, A., 2009b. Factors influencing concentrations of polybrominated diphenyl ethers (PBDEs) in students from Antwerp, Belgium. Environ. Sci. Technol. 43, 3535-3541.

Roze, E., Meijer, L., Bakker, A., Van Braeckel, K.N.J.A., Sauer, P.J.J., Bos, A.F., 2009. Prenatal Exposure to Organohalogens, Including Brominated Flame Retardants, Influences Motor, Cognitive, and Behavioral Performance at School Age. Environ. Health Persp. 17, 1953-1958.

Saito, I., Onuki, A., Seto, H., 2007. Indoor organophosphate and polybrominated flame retardants in Tokyo. Indoor Air 17, 28-36.

Schecter, A., Päpke, O., Joseph, J.E., Tung, K.C., 2005. Polybrominated diphenyl ethers (PBDEs) in U.S. computers and domestic carpet vacuuming: Possible sources of human exposure. J. Toxicol. Environ. Health A 68, 501-513.

Shaw, S.D., Blum, A., Weber, R., Kannan, K., Rich, D., Lucas, D., Koshland, C.P., Dobraca, D., Hanson, S., Birnbaum, L.S., 2010. Halogenated Flame Retardants: Do the Fire Safety Benefits Justify the Risks? Rev. Environ. Health 25, 261-305.

Shoeib, M., Harner, T., Webster, G.M., Sverko, E., Cheng, Y., 2012. Legacy and current-use flame retardants in house dust from Vancouver, Canada. Environ. Pollut. 196, 175-182.

Sjödin, A., Päpke, O., McGahee, E., Focant, J.-F., Jones, R.S., Pless-Mulloli, T., Toms, L.-M.L., Herrmann, T., Müller, J., Needham, L.L., Patterson Jr, D.G., 2008. Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries. Chemosphere 73, S131-S136.

Stapleton, H.M., Dodder, N.G., Offenberg, J.H., Schantz, M.M., Wise, S.A., 2005. Polybrominated diphenyl ethers in house dust and clothes dryer lint. Environ. Sci. Technol. 39, 925-931.

Stapleton, H.M., Eagle, S., Sjödin, A., Webster, T.F., 2012. Serum PBDEs in a North Carolina toddler cohort: Associations with handwipes, house dust, and socioeconomic variables. Environ. Health Persp. 120, 1049-1054.

Stockholm Convention, 2009. The new POPs under the Stockholm Convention. https://chm.pops.int/Home/Loginpage/tabid/307/Default.aspx?returnurl=%2fProgrammes%2fNewPOPs%2fThe9newPOPs%2ftabid%2f672%2flanguage%2fen-US%2fDefault.aspx#LiveContent[4-5-BDE]. (accessed December, 2013).

Stockholm Convention, 2011. Risk management evaluation on hexabromocyclododecane Persistent Organic Pollutants Review Committee.

Takigami, H., Suzuki, G., Hirai, Y., Sakai, S.i., 2009. Brominated flame retardants and other polyhalogenated compounds in indoor air and dust from two houses in Japan. Chemosphere 76, 270-277.

Tang, L., Lei, B., Xu, G., Ma, J., Lei, J.Q., Jin, S.Q., Hu, G.Y., Wu, M.H., 2013. Polybrominated Diphenyl Ethers in Human Hair from the College Environment: Comparison with Indoor Dust. B. Environ. Contam. Tox. 91, 377-381.

Thuresson, K., Hoglund, P., Hagmar, L., Sjodin, A., Bergman, A., Jakobsson, K., 2006. Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. Environ. Health Perspect. 114, 176-181.

Toms, L.-M.L., Hearn, L., Kennedy, K., Harden, F., Bartkow, M., Temme, C., Mueller, J.F., 2009a. Concentrations of polybrominated diphenyl ethers (PBDEs) in matched samples of human milk, dust and indoor air. Environ. Int. 35, 864-869.

Toms, L.M.L., Bartkow, M.E., Symons, R., Paepke, O., Mueller, J.F., 2009b. Assessment of polybrominated diphenyl ethers (PBDEs) in samples collected from indoor environments in South East Queensland, Australia. Chemosphere 76, 173-178.

Trudel, D., Scheringer, M., Von Goetz, N., Hungerbühler, K., 2011. Total consumer exposure to polybrominated diphenyl ethers in north america and europe. Environ. Sci. Technol. 45, 2391-2397.

US-EPA, 2011. Exposure Factors Handbook: 2011 Edition. National Center for Environmental Assessment, Washington, DC, National Technical Information Service.

US-EPA, 2013. Biominitoring: Polybrominated Diphenyl Ethers (PBDEs). America's Children and the Environment | Third Edition, pp. 159-167.

van der Ven, L.T., Verhoef, A., van de Kuil, T., Slob, W., Leonards, P.E., Visser, T.J., Hamers, T., Herlin, M., Hakansson, H., Olausson, H., Piersma, A.H., Vos, J.G., 2006. A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats. Toxicol. Sci. 94, 281-292.

Vorkamp, K., Thomsen, M., Frederiksen, M., Pedersen, M., Knudsen, L.E., 2011. Polybrominated diphenyl ethers (PBDEs) in the indoor environment and associations with prenatal exposure. Environ. Int. 37, 1-10.

Watkins, D.J., McClean, M.D., Fraser, A.J., Weinberg, J., Stapleton, H.M., Sjödin, A., Webster, T.F., 2011. Exposure to PBDEs in the Office Environment: Evaluating the Relationships Between Dust, Handwipes, and Serum. Environ Health Perspect 119.

Wei, H., Turyk, M., Cali, S., Dorevitch, S., Erdal, S., Li, A., 2009. Particle size fractionation and human exposure of polybrominated diphenyl ethers in indoor dust from Chicago. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 44, 1353-1361.

Whitehead, T., Metayer, C., Buffler, P., Rappaport, S.M., 2011. Estimating exposures to indoor contaminants using residential dust. J. Expos. Sci. Environ. Epidemiol. 21, 549-564.

WHO/UNEP, 2013. State of the Science of Endocrine Disrupting Chemicals 2012 - Summary for Decision-Makers. in: Bergman, Å., Heindel, J.J., Jobling, S., Kidd, K.A., Zoeller, R.T. (Eds.).

Wilford, B.H., Shoeib, M., Harner, T., Zhu, J., Jones, K.C., 2005. Polybrominated Diphenyl Ethers in Indoor Dust in Ottawa, Canada: Implications for Sources and Exposure. Environ. Sci. Technol. 39, 7027-7035.

Wong, F., Cousins, I.T., MacLeod, M., 2013. Bounding uncertainties in intrinsic human elimination half-lives and intake of polybrominated diphenyl ethers in the North American population. Environ. Int. 59, 168-174.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., La Guardia, M., McClean, M.D., Webster, T.F., 2007. Human Exposure to PBDEs: Associations of PBDE Body Burdens with Food Consumption and House Dust Concentrations. Environ. Sci. Technol. 41, 1584-1589.

Yu, Y., Yang, D., Wang, X., Huang, N., Zhang, X., Zhang, D., Fu, J., 2013. Factors influencing on the bioaccessibility of polybrominated diphenyl ethers in size-specific dust from air conditioner filters. Chemosphere 93, 2603–2611.

Zhang, X., Diamond, M.L., Robson, M., Harrad, S., 2011. Sources, Emissions, and Fate of Polybrominated Diphenyl Ethers and Polychlorinated Biphenyls Indoors in Toronto, Canada. Environ. Sci. Technol. 45, 3268–3274.

Zheng, J., Luo, X.-J., Yuan, J.-G., Wang, J., Wang, Y.-T., Chen, S.-J., Mai, B.-X., Yang, Z.-Y., 2011. Levels and sources of brominated flame retardants in human hair from urban, e-waste, and rural areas in South China. Environ. Pollut. 159, 3706-3713.

Zoeller, R.T., Brown, T.R., Doan, L.L., Gore, A.C., Skakkebaek, N.E., Soto, A.M., Woodruff, T.J., Vom Saal, F.S., 2012. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. Endocrinology 153, 4097-4110.

Zota, A.R., Rudel, R.A., Morello-Frosch, R.A., Brody, J.G., 2008. Elevated house dust and serum concentrations of PBDEs in California: Unintended consequences of furniture flammability standards? Environ. Sci. Technol. 42, 8158-8164.

3.2. Brominated, chlorinated and phosphate organic contaminants in house dust from Portugal

Abstract

House dust is an important matrix to evaluate the human exposure to a large number of contaminants including organochlorine compounds and flame retardants. In this study, we measured the levels of polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDDs), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), decabromodiphenyl ethane (DBDPE), polychlorinated biphenyls (PCBs) and several organophosphorus flames retardants (PFRs) in 28 house dust samples collected between 2010 and 2011 in two Portuguese cities, Aveiro and Coimbra. Among the measured compounds, PFRs, particularly tris(2-ethylhexyl) phosphate (TEHP), triphenyl phosphate (TPHP), 2-ethylhexyl diphenyl phosphate (EHDPP) and tris(methylphenyl) phosphate (TMPP), were the dominant group (median: 3200 ng g^{-1}). PBDE levels were the second highest (median: 340 ng g^{-1}) with great predominance of BDE 209 (median 270 ng g^{-1}), followed by HBCDDs (median: 150 ng g^{-1}), DBDPE (54 ng g^{-1}), PCBs (median: 6.3 ng g^{-1}) and BTBPE (median: 1.2 ng g^{-1}). Estimated daily intakes (EDIs) via dust ingestion showed a higher intake of PFRs (median: 4.6 ng kg-bw^{-1} day ^{1}); however for all contaminants the EDIs were much lower than the established reference dose (RfD) values. Therefore, the studied population is exposed to non-hazardous levels of the target compounds when considering the exposure through house dust ingestion.

Keywords: dust, BFRs, EBFRs, PCBs, PFRs.

3.2.1. Introduction

Indoor dust is a repository of many organic and inorganic contaminants (Butte and Heinzow, 2002). As modern people tend to spend about 85-98% of their time indoors (Liagkouridis et al., 2014) exposure to indoor dust is inevitable. Therefore, house dust has been used to evaluate human exposure to a large number of compounds accumulated indoors.

Polychlorinated biphenyls (PCBs) and flame retardants (FRs) are two typical groups of compounds detected in house dust known to be hazardous to human health. FRs have been applied in various products that contain potentially flammable materials in order to reduce the devastating impact of fires. They have the ability to minimize the flame spread and the generation of smoke by slowing the combustion of polymers (Fromme et al., 2014). However, the provided safety benefits have currently been side by side with the concern of environmental contamination, toxicity and deleterious effects to animal and human health. About 200 distinct chemicals are used as FRs (Webster and Stapleton, 2012), these chemicals exhibit different structures and properties and can be inorganic or organic. This latter category includes organohalogenated and organophosphorus compounds.

PCBs unique properties such as chemical stability, high boiling point, low heat conductivity and high dielectric constants, led to their large industrial and commercial applicability (EFSA, 2005). Due to their non-flammability they were also manufactured as FRs from the late 1920s to the mid-1980s (Bergman et al., 2012), though the main application of PCBs was as dielectric oil in the transformers and capacitors. Since PCBs are classified as persistent organic pollutants (POPs), their usage was banned in the majority of industrial countries, hence they were substituted by other chemicals (EFSA, 2010).

Brominated flame retardants (BFRs), alongside chlorinated flame retardants (CFRs), are included in the group of halogenated compounds (Bergman et al., 2012). BFRs include polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD) and emerging brominated flame retardants (EBFRs). PBDEs and HBCDDs stand out among the BFRs detected in the environment. However, the application of the three major commercial mixtures of PBDEs has already been banned in many countries.

In 2004, the production of penta-BDE and octa-BDE mixtures was discontinued in Europe and United States (US). Four years later, the application of deca-BDE mixtures in electric and electronic devices was phased out in Europe and their use was completely banned in the US in 2013. HBCDDs commercialization was banned in August 2015 in Europe (Coelho et al., 2014).

As a consequence of these restrictions, some alternatives were introduced into the market including EBFRs and organophosphorus flame retardants (PFRs), thereby the demand for these FRs has been increasing in the last several years. For instance, the EBFRs, 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) and decabromodiphenyl ethane (DBDPE) arise as replacements for octa-BDEs and deca-BDEs, respectively (Newton et al., 2015).

Additionally, the consumption of PFRs has been growing considerably; in 2006 the European consumption of FRs was 465,000 tons of which 10% were BFRs and 20% were PFRs (van der Veen and de Boer, 2012). Similarly to BFRs, they are used in the manufacture of different products, such as plastics, polyurethane foams, thermosets, coatings and textiles. PFRs are divided into two main classes; (1) halogenated phosphates such as tris(2-chloroethyl) phosphate (TCEP) and tris (1,3-dichloro- 2-propyl) phosphate TDCIPP, and (2) non-halogenated compounds, tripropyl phosphate (TPP), tri-n-butyl phosphate (TNBP), 2-ethylhexyl diphenyl phosphate (EHDPP), tricresyl phosphate (TMPP), tripentyl phosphate (TPEP), triphenyl phosphate (TPHP) and tris(2-ethylhexyl) phosphate (TEHP).

According to the vast literature published before now, the above-mentioned contaminants are widespread in the environment and are considered to be hazardous to wildlife and human health (e.g. Fromme et al., 2015; IARC, 2015; van der Veen and de Boer, 2012). Considering their toxicity and widespread occurrence in the indoor environment, and the lack of information regarding their indoor levels in Portuguese houses, the present study aims to evaluate the levels of selected FRs (PBDEs, HBCDDs, EBFRs and PFRs) and PCBs in house dust samples from two Portuguese cities (Aveiro and Coimbra).

3.2.2. Materials and Methods

3.2.2.1. Collection of house dust samples

Dust sampling was carried out in two cities from central Portugal: Aveiro and Coimbra. Between February 2010 and November 2011, volunteers from Aveiro (n=18) and from Coimbra (n=10) were recruited by convenience and agreed to participate in the sampling campaign by signing an informed consent. The participants were asked to answer a questionnaire and to provide the vacuum cleaner bags that were currently in use to clean their respective residences. Hence, the bags collected in the different houses might correspond to different sampling periods (mean 90 days). In the surveyed houses all rooms were regularly vacuumed and therefore the dust samples collected reflect the contamination of the entire house. Housing characteristics, in which dust samples were collected, are described in Table 3.2.1. In our laboratory, house dust was removed from each vacuum cleaner bag and following the procedure described by similar studies (e.g. Ali et al., 2012b; Björklund et al., 2012; Brommer et al., 2012; Dirtu et al., 2012; Kim et al., 2013; Roosens et al., 2009; Van den Eede et al., 2012; Van den Eede et al., 2011), the sample was sieved through a stainless steel sieve (500 μm) to remove fibrous materials and large pieces in order to obtain a suitable degree of homogeneity. The samples were kept in clean amber glass vials and stored at -25 °C in the Environmental Specimen Bank (es-BANK) of Ehime University until chemical analysis.

3.2.2.2. Chemical analysis

Details of the analytical methods for BFRs, EBFRs, PCBs and PFRs were reported elsewhere (Asante et al., 2013; Kim et al., 2013).

3.2.2.2.1. BFRs, EBFRs and PCBs

About 2 g of each sieved dust sample were mixed with anhydrous sodium sulfate and extracted with a high speed solvent extractor (SE-100, Mitsubishi Chemical Analytech) using an acetone/hexane (1:1 *v/v*) solution. An aliquot of the extract corresponding to 1 g

of dust was spiked with $^{13}C_{12}$-labeled PBDEs and $^{13}C_{12}$-labeled PCBs (5 ng each) and 10 ng of $^{13}C_{12}$-labeled HBCDDs as surrogates.

The spiked portion was treated with sulphuric acid (98%), washed with hexane-washed water, and then run through a multi-layer silica gel column for clean-up. The pre-clean-up solution was subjected to gel permeation chromatography (GPC: Bio-Beads S-X3, Bio-Rad, CA, 2 cm i.d.×50 cm) with a mobile phase of dichloromethane/hexane (1:1*v/v*) solution for further clean-up. The fraction containing organohalogen compounds was concentrated using a rotary evaporator and fractionated through a 4 g of activated silica gel (Wakogel DX) packed in a glass column. First fraction, containing PBDEs, BTBPE, DBDPE and PCBs, was eluted with 80 ml of dichloromethane/hexane (5% *v/v*), and the second, containing HBCDDs, with 100 ml of dichloromethane/hexane (25% *v/v*). To ensure the recoveries of the surrogates, the first fraction was evaporated and spiked with $^{13}C_{12}$-labeled BDE 126 and BDE 205 for PBDE IS and $^{13}C_{12}$-labeled BDE 139 for PCB IS while the second fraction containing HBCDDs was spiked with HBCDD-d_{18} (α-, β-, γ-HBCDD-d_{18}). Identification and quantification of PBDEs, BTBPE, DBDPE and PCBs were performed using a gas chromatograph (GC: Agilent 7890 A) coupled with a mass spectrometer (MS: Agilent 5975 C). GC columns used were DB-1MS (length: 30 m x internal diameter: 0.250 mm x film thickness: 0.25µm, Agilant J&W) for PCBs, mono to hepta BDEs and BTBPE, and DB-5HT (15m x 0.25mm x 0.1µm, Agilant J&W) for octa to deca BDEs and DBDPE.

Considering HBCDD isomers, an ultra-performance liquid chromatograph equipped with a Quattro Micro API triple–quadrupole mass spectrometer (UPLC-MS/MS, Waters/Micromass, Tokyo, Japan) was used for analysis. The separation of the 3 diastereoisomers was achieved with an Agilent Extend C18 (2.1 mm x 100 mm, 1.8 µm) column.

3.2.2.2.2. Organophosphorus flame retardants

Another portion of the extract corresponding to approximately 0.1 g of the house dust was purified with a 6% H_2O deactivated alumina column (1 cm i.d. x 20 cm). The target PFRs were eluted with 50 mL of dichloromethane. After complete solvent evaporation, the

analytes were reconstituted in 1 mL of methanol (MeOH), and 1 ng of TPP-d15 was added as an internal standard.

Nine PFRs were identified and quantified using an UPLC (UFLC-XR, Shimadzu Corporation, Japan) coupled with an AB Sciex 5500 Q-Trap electrospray triple-quadrupole mass spectrometer (AB Sciex, Foster City, CA) in positive ionization mode. A 10 μL aliquot of final MeOH solution containing PFRs was injected onto an Asentis Express C18 analytical column (2.7 μm, 2.1 mm i.d. x 100 mm; Supelco, Bellefonte, PA) and separated with the mobile phase of 0.1% (v/v) formic acid in Milli-Q water (A) and 10 mM ammonium acetate in methanol (B) at a flow rate of 0.2 mL/min. The gradient conditions were as follows; (A) 80% + (B) 20 % as initial conditions and held for 2 min, (A) 5% + (B) 95% at 3 min and held for 8 min, and (A) 0% + (B) 100% at 9 min and held for 13 min. The MS/MS parameters for the instrument were optimized for individual analytes by the protocol reported previously (Kim et al., 2011).

3.2.2.3. Quality control and quality assurance

For quality assurance and quality control (QA/QC), with every batch of samples, a laboratory blank was analysed in order to assess possible contamination during the analytical procedure. The limits of detection (LODs) were calculated as three times the standard deviation of background peaks in the procedural blanks. LODs were: 0.23 ± 0.007 ng g^{-1} for BDE 28, 47, 99, 100, 153, 154 and 183, HBCDDs and BTBPE; 0.91 ± 0.030 ng g^{-1} for BDE 209 and DBDPE; between 0.15 ± 0.005 ng g^{-1} and 0.51 ± 0.017 ng g^{-1} for non-dioxin-like PCBs; between 0.27 ± 0.009 ng g^{-1} and 0.62 ± 0.020 ng g^{-1} for dioxin-like PCBs; and 0.86 ± 0.023 ng g^{-1}, 0.44 ± 0.028 ng g^{-1}, 1.5 ± 0.031 ng g^{-1}, 0.84 ± 0.011 ng g^{-1}, 0.28 ± 0.012 ng g^{-1}, 1.8 ± 0.019 ng g^{-1}, 1.5 ± 0.010 ng g^{-1}, 4.0 ± 0.029 ng g^{-1} and 1.8 ± 0.014 ng g^{-1} for TPP, TNBP, EHDPP, TMPP, TPEP, TPHP, TEHP, TCEP and TDCIPP, respectively. Concentrations below the limit of detection (LOD) were considered as zero for all calculations.

Recoveries of the surrogates varied between 78 and 136 % for PBDEs, 76 and 89 % for HBCDDs, 87 and 92 % for BTBPE, between 161 and 200 % for DBDPE, 59 and 90 % for PCBs and 76 and 117% for PFRs.

3.2.2.4. Statistical analysis

Data regarding the general statistical description on the levels of target compounds in the analyzed samples were calculated through the Microsoft Office Excel 2013. Spearman rank correlation (for continuous variables) and Kruskal-Wallis test (for nominal variables) were used to assess the associations between selected house characteristics (information gathered from the questionnaire) and the levels of BFRs, PCBs and PFRs. These analyses were performed using IBM SPSS Statistics 20. Sigma Plot V11.0 was used for the box-plot graphs on the concentration distribution of BFRs, PCBs, and PFRs (*i.e.*, Figure 3.2.1). All statistical analyses were carried out for a significance level of 0.05.

3.2.2.5. Estimation of human exposure/ Calculation of daily intake

Human exposure through dust ingestion was evaluated by calculating the estimated daily intakes (EDIs) of the target compounds, based on the high dust ingestion rates of 100 mg day^{-1} for adults and 200 mg day^{-1} for children (Wilford et al., 2005), which reflect the worst case scenarios.

The calculations were based on an average body weight of 70 kg for adults and 12 kg for children (EFSA, 2012).

3.2.3. Results and discussion

3.2.3.1. Housing characteristics

Dust samples were collected from 28 houses located in Aveiro and Coimbra, which were mainly apartments (60.7%) located in the urban area (64.3%) (Table 3.2.1). Most of the surveyed houses were built after 2000 (60.7%) and their areas were highly variable (Table 3.2.1).

Table 3.2.1. Housing characteristics (n=28).

Characteristics		% of houses (n)[a]
District	Aveiro	64.3 (18)
	Coimbra	35.7 (10)
Construction year	< 1990	21.4 (6)
	1990 - 1994	7.1 (2)
	1995 - 1999	3.6 (1)
	2000 - 2004	32.1 (9)
	≥ 2005	28.6 (8)
House area (m^2)	< 30	3.6 (1)
	30 - 129	42.9 (12)
	130 - 229	28.6 (8)
	230 - 329	17.9 (5)
	≥ 330	3.6 (1)
Location	Urban	64.3 (18)
	Sub-urban	17.9 (5)
	Agricultural	14.3 (4)
House Type	Apartment	60.7 (17)
	Private House	35.7 (10)
No. of electronic devices[b]	< 5	25.0 (7)
	5 - 9	57.1 (16)
	≥ 10	14.3 (4)

[a] For one house from Aveiro District no information on housing characteristics was available, furthermore for another house also from Aveiro the information on the construction year was not provided.
[b] Including TVs, computers, stereos, VCRs and DVD players.

3.2.3.2. Concentrations in house dust

3.2.3.2.1. BFRs

Concentrations of analyzed BFRs are listed in Table 3.2.2. For PBDEs, the total and primary congener levels (BDE 28, 47, 99, 100, 153, 154, 183, and 209) varied from 38 to 9600 ng g^{-1} (median: 340 ng g^{-1}) and from 34 to 9200 ng g^{-1} (median: 300 ng g^{-1}), respectively. BDE 47, 99 and 209 were detected in all the 28 dust samples being the main contributors for the total PBDE concentration. Such predominance of BDE 47, 99 and 209 is in accordance with previous results from dust samples of different origins (houses, cars, public places and offices) collected around the world (see review by Coelho et al. (2014)). A previous study on house dust samples collected in Porto region, Portugal also showed BDE 209 as the most abundant congener followed by BDE 47 and BDE 99 (Cunha et al.,

2010). However, the median concentrations of BDE 209 (950 ng g^{-1}) and BDE 47 (19 ng g^{-1}) reported by Cunha et al. (2010) were slightly higher than those observed in this study (270 ng g^{-1} for BDE 209 and 5.7 ng g^{-1} for BDE 47), with the exception of BDE 99 that showed similar concentrations (medians about 6 ng g^{-1} each). Elevated levels of BDE 209 are in accordance with other European studies (e.g. Björklund et al., 2012; Newton et al., 2015; Sahlström et al., 2015). These results may reflect a greater use of deca-BDE commercial formulation containing mainly BDE 209 than the penta-BDE mixture composed primarily of BDE 47, 99 and 100. Another possible reason might be the due to BDE 209 instability in the products and strong affinity to particles (UNEP, 2014), which potentiates its migration from the consumer products and to adsorb to the indoor dust.

Table 3.2.2. Concentrations (ng g^{-1}) and detection frequency (DF %) of BFRs in the 28 dust samples analyzed.

	DF % (n)	Mean	Median	Range
BDE 28	32.1 (9)	0.35	< 0.23	< 0.23 – 2.1
BDE 47	100 (28)	12	5.7	1.3 – 110
BDE 99	100 (28)	12	6.3	1.4 – 58
BDE 100	78.6 (22)	2.2	1.2	< 0.23 – 14
BDE 153	57.1 (16)	1.3	0.75	< 0.23 – 7.7
BDE 154	35.7 (10)	0.64	< 0.23	< 0.23 – 3.6
BDE 183	89.3 (25)	5.2	2.4	< 0.23 – 70
BDE 209	100 (28)	780	270	24 – 9200
Σ PBDEs[a]	_	890	340	38 – 9600
Σ tri-hepta-BDE[b]	_	34	16	4.5 – 190
Σ 8 PBDEs[c]	_	810	300	34 – 9200
α-HBCDD	100 (28)	200	91	11 – 1800
β-HBCDD	100 (28)	43	16	1.9 – 230
γ-HBCDD	100 (28)	130	35	2.8 – 900
Σ HBCDDs	_	380	150	16 – 2000
BTBPE	50 (14)	13	1.2	< 0.23 – 96
DBDPE	100 (28)	60	54	12 – 150

[a] Sum of all congeners; [b] Sum of BDE 28, 47, 99, 100, 153, 153 and 183; [c] Sum of BDE 28, 47, 99, 100, 153, 153, 183 and 209.
(n): number of samples in which BFRs were detected.

Although previous studies that analyzed dust samples from the United States (US) have generally disclosed higher levels of BDE 209 than those reported in Europe, the large differences among the levels of BDE 209 and BDE 47 and 99 observed in Europe (well characterized by Sjödin et al. (2008)) were not observed in the US dust (e.g. Dodson et al., 2012; Schreder and La Guardia, 2014; Whitehead et al., 2013). These small differences are

probably due to the fact that the penta-BDE mixture was extensively consumed in North America (EFSA, 2011).

Total concentrations of HBCDDs varied between 16 and 2000 ng g^{-1}, and the three HBCDD isomers were detected in all the dust samples. α-HBCDD exhibited the highest median concentration, 91 ng g^{-1}, which accounted for 79% of the total concentration (median: 150 ng g^{-1}), followed by γ-HBCDD (median: 35 ng g^{-1}) and β-HBCDD (median: 16 ng g^{-1}). This HBCDD isomer profile does not follow the general global pattern of HBCDDs in indoor dust, γ-HBCDD > α-HBCDD > β-HBCDD, described by Coelho et al. (2014), which reflects the composition of the HBCDD commercial mixture. Nevertheless, some surveys also described α-HBCDD as the dominant isomer, as for example in house dust from Belgium, Germany, Czech Republic, Sweden and from the United States (Roosens et al., 2009, Fromme et al., 2014, Lankova et al., 2015, Sahlström et al., 2015 and Schreder and La Guardia, 2014). This α-HBCDD dominance might result from the thermal treatment of materials during production process. At temperatures above 100ºC, γ-HBCDD might be converted to α-HBCDD (Heeb et al., 2008), which is relatively stable and bioaccumulative (Kuo et al., 2014). Furthermore, it is well known that, in Europe, HBCDDs used in certain plastics or fabrics are treated or pressed with a thermal process. Therefore, although γ-HBCDD is more abundant in the commercial formulation, α-HBCDD can be dominant in environmental samples, including dust.

The median concentrations of Σ HBCDDs in the aforementioned studies are in the same range (ca 100 ng g^{-1}) as our study (150 ng g^{-1}), with the exception of German and American samples that exhibited median values for Σ HBCDDs two or more times higher (345 and 300 ng g^{-1}, respectively) (Fromme et al., 2014; Schreder and La Guardia, 2014).

Concerning EBFRs, while the detection frequency of BTBPE was 50% with levels varying from < 0.23 to 96 ng g^{-1} (median: 1.2 ng g^{-1}), DBDPE was detected in all samples with concentrations between 12 and 150 ng g^{-1} (median: 54 ng g^{-1}). These concentrations are in the same order of magnitude as those detected in New Zealand dust samples with median concentrations of 2 and 23 ng g^{-1} for BTBPE and DBDPE, respectively (Ali et al., 2012a). In house dust, generally, BTBPE displays lower concentrations when comparing with DBDPE. In fact, similarly to our survey, dust samples from Czech Republic (Kalachova et al., 2012), Sweden (Sahlström et al., 2015) and the United States (Dodson et al., 2012) exhibited lower levels of BTBPE (with median values of < 2; 6.3 and 12 ng g^{-1},

respectively) when compared with the median concentrations of DBDPE in the same samples (141, 150 and 140 ng g^{-1}, respectively).

Overall, among the BFRs targeted, PBDEs were dominant in Portuguese house dust, followed by HBCDDs and EBFRs (Table 3.2.2 and Figure 3.2.1); however when excluding the BDE 209 congener, HBCDDs become the major BFR group. Similar trends were observed in Romanian indoor dust samples (Dirtu et al., 2012).

3.2.3.2.2. PCBs

Generally, PCBs were detected in house dust samples at lower levels than BFRs (Figure 3.2.1), with the total PCBs concentrations varying from 0.18 to 61 ng g^{-1} (summarized in Table 3.2.3). Non-dioxin-like PCBs (ndl-PCBs) were the predominant congeners, particularly PCB 138, 101, 153 and 180 with DFs of 85.7, 89.3, 96.4 and 100%, respectively. These results might reflect the predominant use of Aroclor 1254 and 1260 commercial formulations, which were widely used in Europe (Cachada et al., 2009). These results are in accordance with other studies that analyzed house dust samples from Belgium (Roosens et al., 2010), United States, Canada and New Zealand (Harrad et al., 2009), Romania (Dirtu et al., 2012), Singapore (Tan et al., 2007b) and Pakistan (Ali et al., 2012b).

In this study, however, PCB 28 also exhibited high detection frequencies (75%), and thus having an impact on total PCBs concentrations. PCB 28 was the main congener found in dust samples from the United Kingdom, New Zealand (Harrad et al., 2009) and Germany (Abb et al., 2010). This congener is integrated in the commercial Aroclor 1242 that is mainly constituted by tri- and tetrachlorinated PCBs.

Dioxin-like PCBs showed median concentrations below the limit of detection (LOD) for all the congeners. In contrast, Hinwood et al. (2014) measured the levels of dioxin-like PCBs in house dust samples from Australia and all congeners showed median concentrations over the LOD with the exception of PCB 169. PCB 118 showed the highest concentration, followed by PCB 156 and PCB 105 (Hinwood et al., 2014).

The majority of the previous surveys in other countries reported higher total concentrations of PCBs than our study (median: 6.3 ng g^{-1}), with the exception of two surveys conducted

in Singapore (median: 5.6 ng g^{-1} (Tan et al., 2007b)) and Pakistan (median: 0.67 ng g^{-1} (Ali et al., 2012b)), implying less PCB contamination in Portuguese indoor environment.

Table 3.2.3. Concentrations (ng g^{-1}) and detection frequency (DF %) of PCBs in the 28 dust samples analyzed.

	DF % (n)	Mean	Median	Range
Non-dioxin-like PCBs				
PCB 28	75 (21)	0.59	0.62	< 0.3 – 2.8
PCB 52	39.3 (11)	< 0.51	< 0.51	< 0.51 – 1.4
PCB 101	89.3 (25)	1.3	0.68	< 0.31 – 6.0
PCB 138	85.7 (24)	1.7	0.87	< 0.35 – 9.7
PCB 153	96.4 (27)	1.7	0.86	< 0.24 – 9.6
PCB 180	100 (28)	0.95	0.66	0.18 – 4.3
Dioxin-like PCBs*				
PCB 77	3.6 (1)	< 0.52	< 0.52	< 0.52 – 0.58
PCB 105	28.6 (8)	< 0.31	< 0.31	< 0.31 – 3.2
PCB 118	35.7 (10)	0.65	< 0.52	< 0.52 – 5.7
PCB 156	14.3 (4)	< 0.41	< 0.41	< 0.41 – 0.82
PCB 167	3.6 (1)	< 0.33	< 0.33	< 0.33 – 0.35
Σ ndl-PCBs[a]	_	6.4	3.7	0.18 – 30
Σ dl-PCBs[b]	_	1.0	< LOD[§]	< LOD[§] – 9.7
Σ 18 PCBs[c]	_	7.5	3.9	0.18 – 35
ΣPCBs	_	13	6.3	0.18 – 61

[a] sum of non-dioxin-like PCBs (28, 52, 101, 138, 153 and 180); [b] dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189); [c]sum of dioxin-like PCBs and non-dioxin-like PCBs.
*PCB 81, 114, 123, 126, 157, 169 and 189 were not included since they were not detected (< LOD) in all samples.
(n): number of samples in which PCBs were detected.
[§]LOD: limit of detection; LODs between 0.27 and 0.62 ng g^{-1}

3.2.3.2.3. PFRs

Concentrations of nine PFR compounds detected in house dust are summarized in Table 3.2.4. Compared to BFRs and PCBs, PFRs were the most abundant compounds in the surveyed houses with concentrations ranging from 920 to 35000 ng g^{-1} (median: 3200 ng g^{-1}). The median concentration of PFRs was one order of magnitude higher than PBDEs (median: 340 ng g^{-1}) and HBCDDs (median: 150 ng g^{-1}) and three orders of magnitude higher than PCBs (median: 6.3 ng g^{-1}) (Figure 3.2.1).

Table 3.2.4. Concentrations (ng g^{-1}) and detection frequency (DF %) of PFRs in the 28 dust samples analyzed.

	DF % (n)	Mean	Median	Range
TPP	75 (21)	3.0	2.4	< 0.86 – 10
TNBP	92.9 (26)	73	28	< 0.44 – 550
EHDPP	100 (28)	1100	620	150 – 7200
TMPP	100 (28)	130	97	24 – 480
TPEP	53.6 (15)	2.7	0.75	< 0.28 – 25
TPHP	100 (28)	1000	660	110 – 5200
TEHP	100 (28)	2200	1700	250 – 23 000
TCEP	82.1 (23)	58	17	< 4.0 – 720
TDCIPP	53.6 (15)	60	22	< 1.8 – 390
ΣPFRs	_	4700	3200	920 – 35 000

(n): number of samples in which PFRs were detected.

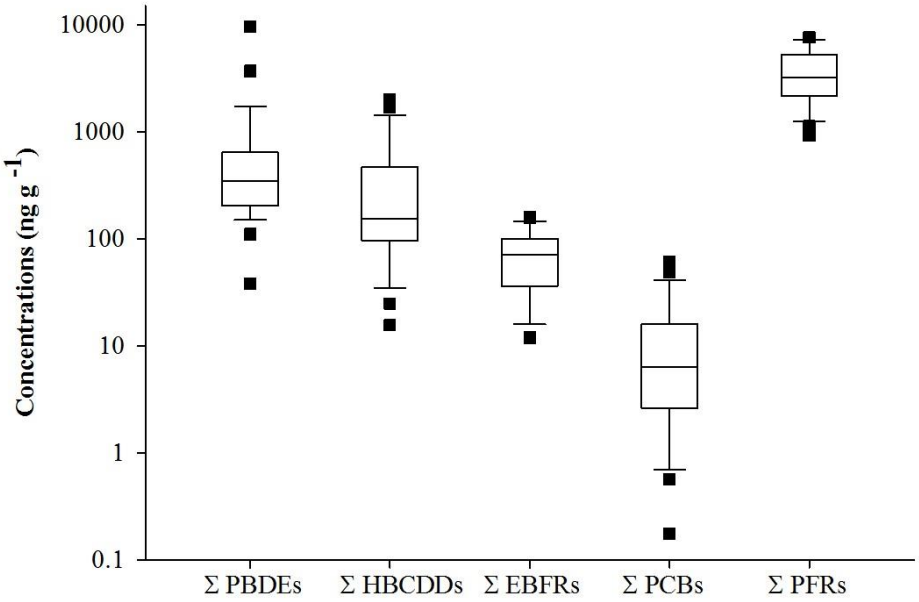

Figure 3.2.1. Boxplot summarizing the variation in total concentrations of the target compounds measured in the 28 house dust samples from Portugal. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).

Median concentrations for all PFR compounds were over the LOD, which indicates their ubiquitous distribution in the Portuguese houses. Non-halogenated PFRs, TEHP, TPHP, EHDPP and TMPP, were detected in all the 28 samples with elevated median levels, 1700, 660, 620 and 97 ng g^{-1}, respectively. Although the number of studies reporting the levels of TEHP in house dust is limited, Kim et al. (2013) detected this compound in indoor dust

samples from two different locations in the Philippines. Despite the lower median concentrations (140 ng g^{-1} and 41 ng g^{-1}, in residential and dumping site areas respectively), TEHP also exhibited high detection frequencies and was the main PFR in dust samples from a residential area (Malate) in Philippines. TPHP concentrations detected in our samples were in the same order of magnitude of the levels reported in other studies (Table 3.2.5), with the exception of the Philippines samples in which TPHP levels are relatively lower (Kim et al., 2013).

Considering EHDPP, the levels found in our samples were higher than those reported by other studies (Table 3.2.5). TMPP in Portuguese dust exhibited the lowest concentrations among the four main non-halogenated PFR compounds. This pattern is in agreement with the results observed in other countries (Table 3.2.5).

Table 3.2.5. Median concentrations (ng g^{-1}) of PFRs in house dust from different locations around the world published since 2010.

References	Country	n	TEHP	TPHP	EHDPP	TMPP
Dirtu et al. (2012)	Romania	47	n.a.	500	n.a.	500
Brommer et al. (2012)	Germany	6 (1 house)	n.a.	380 (mean)	n.a.	94 (mean)
Van den Eede et al. (2012)	Belgium	8	n.a.	400	n.a.	120
Van den Eede et al. (2011)	Belgium	33	n.a.	500	n.a.	240
Kim et al. (2013)	Philippines	37	75	78	70	13
Brandsma et al. (2014)	Netherlands	8	n.a.	820	350	110
Canbaz et al. (2015)*	Sweden	110[a], 110[b]	n.a.	419[a], 631[b]	163[a], 172[b]	192[a], 288[b] (mmp-TMPP)
Mizouchi et al. (2015)	Japan	10	< MDL	820	200	1200
This study	Portugal	28	1700	660	620	97

n.a.: information not available
MDL: method detection limit.
*Dust samples were collected from two different groups: [a] mattresses from the mothers that gave birth to children that developed asthma, [b] mattresses from the mothers that gave birth to healthy children (controls).

3.2.3.3. Association with housing characteristics

Associations between the dust levels of the target compounds (Tables 3.2.2, 3.2.3 and 3.2.4) and the housing characteristics depicted in Table 3.2.1 (District, Construction year, House area, Location, House type, No. of electronic devices) were assessed. Regarding PBDEs and HBCDDs, no statistically significant correlations with the aforementioned characteristics were found, though some associations were expected between these BFRs and the construction year or the number of electronic devices. The lack of correlations might be linked to the possibility that the levels of PBDEs and HBCDDs can be more influenced by the last reconstruction date rather than the year of construction, as suggested by Kalachova et al. (2012). Because the penta- and octa-BDEs commercial mixtures were banned in all applications in the European Union in August 2004, while deca-BDE was banned for use in all electric and electronic applications in July 2008 (BSEF, 2013). Other influencing factors can be considered, as the small sample size and the high variety of the sample sources. Similar results showing no correlations were reported in other studies (e.g. Cunha et al., 2010; Kalachova et al., 2012; Tan et al., 2007a; Wu et al., 2007).

For BTBPE, no significant correlations were observed between the dust levels and all the housing characteristics. However, for DBDPE, statistically significant associations were found between its concentrations and the houses' area (Spearman Correlation, $r= 0.442$; $df= 25$; $p= 0.007$), house type (Kruskal-Wallis test, $H= 8.481$, $p= 0.004$) and location (Kruskal-Wallis test, $H= 6.590$, $p= 0.037$). In general, DBDPE concentrations were higher in smaller houses (lower areas and apartments) and in urban locations (Figures B1, B2 and B3 in Annex B).

No correlation was obtained between PCBs levels in dust and the year of construction. Hinwood et al. (2014), for example, reported that dust concentrations of PCBs increased with the increased age of the houses. However, this trend was not noticeable in our survey, probably because most of the houses (82%) were built after the introduction of PCBs legislation. In Portugal, by 1976 their industrial and commercial use was phased out with the exception of their application in dielectric, heat transmitters and hydraulic fluids, that were totally banned in 1988 with the exception of their use for the maintenance of equipment in service and impossible to replace (Garcia et al., 2015). Statistically significant associations were found between Σ PCBs concentrations in dust and the houses'

area (Spearman Correlation, r= 0.361; df= 25; p= 0.008), and location (Kruskal-Wallis test, H= 6.204, p= 0.045), with higher concentrations in smaller houses and in urban locations (Figure B4, B6, Annex B). Despite the correlation with the areas, no association was found with the house type, though, the Σ PCBs concentrations in house dust tended to be higher in apartments (Figures B5, Annex B), like for DBDPE.

For PFR compounds, no correlations were found between their levels and none of the housing characteristics.

3.2.3.4. Estimated daily intakes

In order to evaluate the exposure to BFRs, PCBs, and PFRs via dust ingestion, the estimated daily intakes (EDIs) were calculated based on the high dust ingestion rates of 100 mg day^{-1} for adults and 200 mg day^{-1} for children (Wilford et al., 2005) and considering 70 and 12 kg as the average body weights of adults and children, respectively (EFSA, 2012). EDIs for the target compounds are shown in Table 3.2.6, alongside with the reference dose (RfD) values described by Van den Eede et al. (2011), Ali et al. (2012b) and Kim et al. (2013).

For BFRs, the median EDIs (adults – children) were 0.49 – 5.7, 0.22 – 2.6, 0.0017 – 0.02 and 0.078 – 0.9 ng kg-bw^{-1} day^{-1} for Σ PBDEs, Σ HBCDDs, BTBPE and DBDPE respectively, disclosing higher intakes of PBDEs and HBCDDs rather than the EBFRs. Despite the fact that all calculations were performed using the high dust ingestion rates which reflects the worst case scenario, the EDIs are much lower (more than 1000 times) than the established RfDs of 7000 ng kg-bw^{-1} day^{-1} for BDE 209, 200 x 10^3 ng kg-bw^{-1} day^{-1} for HBCDDs, 243 x 10^3 ng kg-bw^{-1} day^{-1} for BTBPE and 333 x 10^3 ng kg-bw^{-1} day^{-1} for DBDPE (Table 3.2.6).

As expected from the dust concentrations, the intakes of PCBs through dust ingestion were higher for the ndl-PCBs (0.0053 – 0.062 ng kg-bw^{-1} day^{-1}, adults - children). Similarly to BFRs, the intakes of Σ PCBs (0.009 – 0.11 ng kg-bw^{-1} day^{-1}, adults - children) were much lower than the RfD (1000 ng kg-bw^{-1} day^{-1}).

Considering the main four PFR compounds, the median intakes of TEHP (2.4 - 28 ng kg-bw^{-1} day^{-1}, adults - children) were the highest (Table 3.2.6), though it was about 1000 times lower, in children, and more than 1000 times lower, in adults, than the calculated

RfD based on the value described by Kim et al. (2012). The EDIs of TPHP and TMPP, 0.95 – 11 and 0.14 – 1.6 ng kg-bw^{-1} day^{-1} (adults – children), respectively, were also much lower than the RfD values (7000 and 1300 ng kg-bw^{-1} day^{-1}).

Although the worst case scenario has been considered, all the EDIs were far below the established RfD values in all cases. However, this is only one of the potential exposure pathways and hence, in order to assess the accurate risk, other exposure sources must be considered.

3.2.4. Conclusions

This study disclosed the occurrence of several brominated, chlorinated and phosphate organic contaminants in 28 house dust samples from Aveiro and Coimbra in Portugal. Generally, the flame retardants (BFRs, EBFRs and PFRs) exhibited higher detection frequencies and concentrations than PCBs, being the PFRs the most important indoor contaminants among the flame retardant groups, especially TEHP, TPHP, EHDPP and TMPP. Generally, no significant correlations were found between the levels of these contaminants in dust and the housing characteristics, with the exception of DBDPE and PCBs that exhibited higher concentrations in smaller houses and in urban locations. Although most contaminants were detected in the house dust surveyed, their levels were generally low, compared to other European countries and the US, and the EDIs were far below the established RfD values in all cases. However, it is important to note that the ingestion of dust is only one of the potential exposure pathways and that other exposure sources must be considered together with dust ingestion.

Table 3.2.6. Estimated daily intakes of BFRs, PCBs and PFRs via dust ingestion. Calculated from the house dust concentrations considering the worst case scenarios (dust ingestion of 100 and 200 mg day^{-1} for adults and children, respectively) and the average body weights of 70 kg (adults) and 12 kg (children).

	Adult			**Children**			
	Mean *ng kg-bw^{-1} day^{-1}* *(ng day^{-1})*	**Median** *ng kg-bw^{-1} da^{-1}* *(ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1}* *(ng day^{-1})*	**Mean** *ng kg-bw^{-1} day^{-1}* *(ng day^{-1})*	**Median** *ng kg-bw^{-1} day^{-1}* *(ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1}* *(ng day^{-1})*	**RfD** *ng kg-bw^{-1} day^{-1}*
Σ PBDEs	1.3 (89)	0.49 (34)	0.054 – 14 (3.8 – 960)	15 (180)	5.7 (69)	0.63 – 160 (7.6 – 1900)	(BDE 209: 7000)[f]
Σ tri-hepta-BDE[a]	0.048 (3.4)	0.023 (1.6)	0.0065 – 0.27 (0.45 – 19)	0.56 (6.7)	0.27 (3.3)	0.075 – 3.2 (0.90 – 38)	–
Σ 8 PBDEs[b]	1.2 (81)	0.43 (30)	0.048 – 13 (3.4 – 920)	13 (160)	5 (60)	0.56 – 150 (6.7 – 1800)	–
Σ HBCDDs	0.54 (38)	0.22 (15)	0.022 – 2.9 (1.6 – 200)	6.3 (75)	2.6 (31)	0.26 – 33 (3.1 – 400)	200 000[f]
BTBPE	0.019 (1.3)	0.0017 (0.12)	0 – 0.14 (0 – 9.6)	0.22 (2.7)	0.020 (0.24)	0 – 1.6 (0 – 19)	243 000[g]
DBDPE	0.086 (6)	0.078 (5.4)	0.017 – 0.22 (1.2 – 15)	1 (12)	0.9 (11)	0.2 – 2.6 (2.4 – 31)	333 333[g]
Σ ndl-PCBs[c]	0.0092 (0.64)	0.0053 (0.37)	0.00025 – 0.043 (0.018 – 3)	0.11 (1.3)	0.062 (0.74)	0.003 – 0.5 (0.035 – 6.1)	–
Σ dl-PCBs[d]	0.0015 (0.1)	0 (0)	0 – 0.014 (0 – 0.97)	0.017 (0.21)	0 (0)	0 – 0.16 (0 – 1.9)	–
Σ 18 PCBs[e]	0.011 (0.75)	0.0056 (0.39)	0.00025 – 0.05 (0.018 – 3.5)	0.12 (1.5)	0.066 (0.78)	0.003 – 0.58 (0.035 – 7)	–
ΣPCBs	0.018 (1.3)	0.009 (0.63)	0.00025 – 0.087 (0.018 – 6.1)	0.22 (2.6)	0.11 (1.3)	0.003 – 1 (0.035 – 12)	1000[g]
TEHP	3.2 (220)	2.4 (170)	0.36 – 32 (25 – 2300)	37 (450)	28 (340)	4.2 – 380 (50.0 – 4500)	35 000[h]
TPHP	1.5 (100)	0.95 (66)	0.16 – 7.4 (11 - 520)	17 (200)	11 (130)	1.9 – 86 (23 – 1000)	7000[f]

	Adult			Children			RfD
	Mean *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Median** *ng kg-bw^{-1} da^{-1} (ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Mean** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Median** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	*ng kg-bw^{-1} day^{-1}*
EHDPP	1.5	0.89	0.22 – 10	18	10	2.5 – 120	n.a.
	(110)	(62)	(15 – 720)	(210)	(120)	(30 – 1400)	
TMPP	0.18	0.14	0.034 – 0.69	2.1	1.6	0.40 – 8.1	1300[f]
	(13)	(9.7)	(2.4 – 48)	(25)	(19)	(4.8 – 97)	
ΣPFRs	6.6	4.6	1.3 – 51	78	53	15 – 590	–
	(470)	(320)	(92 – 3500)	(930)	(640)	(180 – 7100)	

[a] Sum of BDE 28, 47, 99, 100, 153, 153 and 183; [b] Sum of BDE 28, 47, 99, 100, 153, 153, 183 and 209; [c] sum of non-dioxin-like PCBs (28, 52, 101, 138, 153 and 180); [d] dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189); [e] sum of dioxin-like PCBs and non-dioxin-like PCBs.
[f] Van den Eede et al. (2011).
[g] Ali et al. (2012b).
[h] Kim et al. (2013).
n.a.: Information not available.

Acknowledgements

The authors wish to acknowledge the volunteers who participated in this study. This work was supported by European Funds through COMPETE and by National Funds through the Portuguese Science Foundation (FCT) within projects PEst-C/MAR/LA0017/2013 and PEst-OE/SAU/UI0709/2014. Ana C. A. Sousa and Sónia D. Coelho acknowledge FCT for the grants SFRH/BPD/65884/2009 and SFRH/ BD/78168/2011 (supported by funding from the Human Potential Operational Programme POPH, inscribed in the National Strategic Reference Framework and partially subsidized by the European Social Fund). This study was partly supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) to a project on Joint Usage/Research Center – Leading Academia in Marine and Environmental Research (LaMer), Ehime University. Further support was provided by Grants-in-Aid for Scientific Research (S: 26220103, A: 25257403, A: 16H01784, B: 25281050) of the Japanese Ministry of Education, Science, Sports, Culture and Technology (MEXT) and Japan Society for the Promotion of Science (JSPS).

Supplementary data

Supplementary data to this subchaper (Figures B1 – B6) can be found in Annex B.

References

Abb, M., Breuer, J.V., Zeitz, C., Lorenz, W., 2010. Analysis of pesticides and PCBs in waste wood and house dust. Chemosphere 81, 488-493.

Ali, N., Dirtu, A.C., Eede, N.V.D., Goosey, E., Harrad, S., Neels, H., t Mannetje, A., Coakley, J., Douwes, J., Covaci, A., 2012a. Occurrence of alternative flame retardants in indoor dust from New Zealand: Indoor sources and human exposure assessment. Chemosphere 88, 1276-1282.

Ali, N., Van den Eede, N., Dirtu, A.C., Neels, H., Covaci, A., 2012b. Assessment of human exposure to indoor organic contaminants via dust ingestion in Pakistan. Indoor Air 22, 200-211.

Asante, K.A., Takahashi, S., Itai, T., Isobe, T., Devanathan, G., Muto, M., Agyakwah, S.K., Adu-Kumi, S., Subramanian, A., Tanabe, S., 2013. Occurrence of halogenated contaminants in inland and coastal fish from Ghana: Levels, dietary exposure assessment and human health implications. Ecotox. Environ. Safe. 94, 123-130.

Bergman, A., Rydén, A., Law, R.J., de Boer, J., Covaci, A., Alaee, M., Birnbaum, L., Petreas, M., Rose, M., Sakai, S., Van den Eede, N., van der Veen, I., 2012. A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals. Environ. Int. 49, 57-82.

Björklund, J.A., Sellström, U., de Wit, C.A., Aune, M., Lignell, S., Darnerud, P.O., 2012. Comparisons of polybrominated diphenyl ether and hexabromocyclododecane concentrations in dust collected with two sampling methods and matched breast milk samples. Indoor Air 22, 279-288.

Brandsma, S.H., de Boer, J., van Velzen, M.J.M., Leonards, P.E.G., 2014. Organophosphorus flame retardants (PFRs) and plasticizers in house and car dust and the influence of electronic equipment. Chemosphere 116, 3-9.

Brommer, S., Harrad, S., Van Den Eede, N., Covaci, A., 2012. Concentrations of organophosphate esters and brominated flame retardants in German indoor dust samples. J. Environ. Monit. 14, 2482-2487.

BSEF, 2013. Bromine Science and Environmental Forum – Regulation. http://bsef.com/regulation. (accessed December, 2013).

Butte, W., Heinzow, B., 2002. Pollutants in house dust as indicators of indoor contamination. Rev. Environ. Contam. T. 175, 1-46.

Cachada, A., Lopes, L.V., Hursthouse, A.S., Biasioli, M., Grčman, H., Otabbong, E., Davidson, C.M., Duarte, A.C., 2009. The variability of polychlorinated biphenyls levels in urban soils from five European cities. Environ. Pollut. 157, 511-518.

Canbaz, D., van Velzen, M.J., Hallner, E., Zwinderman, A.H., Wickman, M., Leonards, P.E., van Ree, R., van Rijt, L.S., 2015. Exposure to organophosphate and polybrominated diphenyl ether flame retardants via indoor dust and childhood asthma. Indoor Air [Ahead of print].

Coelho, S.D., Sousa, A.C.A., Isobe, T., Tanabe, S., Nogueira, A.J.A., 2014. Flame retardants in indoor dust - A review on the levels of polybrominated diphenyl ethers and hexabromocyclododecanes. Curr. Org. Chem. 18, 2218-2230.

Cunha, S.C., Kalachova, K., Pulkrabova, J., Fernandes, J.O., Oliveira, M.B.P.P., Alves, A., Hajslova, J., 2010. Polybrominated diphenyl ethers (PBDEs) contents in house and car dust of Portugal by pressurized liquid extraction (PLE) and gas chromatography-mass spectrometry (GC-MS). Chemosphere 78, 1263-1271.

Dirtu, A.C., Ali, N., Van den Eede, N., Neels, H., Covaci, A., 2012. Country specific comparison for profile of chlorinated, brominated and phosphate organic contaminants in indoor dust. Case study for Eastern Romania, 2010. Environ. Int. 49, 1-8.

Dodson, R.E., Perovich, L.J., Covaci, A., Van Den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., Rudel, R.A., 2012. After the PBDE phase-out: A broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol. 46, 13056-13066.

EFSA, 2005. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the presence of non dioxin-like polychlorinated biphenyls (pcb) in feed and food. EFSA J. 1 - 137.

EFSA, 2010. Results of the monitoring of non dioxin-like PCBs in food and feed. EFSA J. 35.

EFSA, 2011. Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA J. 274.

EFSA, 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J. 32.

Fromme, H., Becher, G., Hilger, B., Völkel, W., 2015. Brominated flame retardants – Exposure and risk assessment for the general population. Int. J. Hyg. Environ. Health 219, 1-23.

Fromme, H., Hilger, B., Kopp, E., Miserok, M., Völkel, W., 2014. Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and "novel" brominated flame retardants in house dust in Germany. Environ. Int. 64, 61-68.

Garcia, J.S., Pimpão, A., Baltazar, L., Vilão, R., 2015. Inventário Nacional de PCB - Ano de 2014 Agência Portuguesa do Ambiente, I.P. Departamento de Resíduos / Divisão de Responsabilidade Ambiental e Solos Contaminados pp. 3-23.

Harrad, S., Ibarra, C., Robson, M., Melymuk, L., Zhang, X., Diamond, M., Douwes, J., 2009. Polychlorinated biphenyls in domestic dust from Canada, New Zealand, United Kingdom and United States: Implications for human exposure. Chemosphere 76, 232-238.

Heeb, N.V., Bernd Schweizer, W., Mattrel, P., Haag, R., Gerecke, A.C., Schmid, P., Zennegg, M., Vonmont, H., 2008. Regio- and stereoselective isomerization of hexabromocyclododecanes (HBCDs): Kinetics and mechanism of γ- to α-HBCD isomerization. Chemosphere 73, 1201-1210.

Hinwood, A.L., Callan, A.C., Heyworth, J., Rogic, D., de Araujo, J., Crough, R., Mamahit, G., Piro, N., Yates, A., Stevenson, G., Odland, J.O., 2014. Polychlorinated biphenyl (PCB) and dioxin concentrations in residential dust of pregnant women. Environ. Sci. Process. Impacts 16, 2758-2763.

IARC, 2015. Polychlorinated Biphenyls and Polybrominated Biphenyls. IARC monographs on the evaluation of carcinogenic risks to humans, pp. 513.

Kalachova, K., Hradkova, P., Lankova, D., Hajslova, J., Pulkrabova, J., 2012. Occurrence of brominated flame retardants in household and car dust from the Czech Republic. Sci. Total. Environ. 441, 182-193.

Kim, J.-W., Ramaswamy, B.R., Chang, K.-H., Isobe, T., Tanabe, S., 2011. Multiresidue analytical method for the determination of antimicrobials, preservatives, benzotriazole UV stabilizers, flame retardants and plasticizers in fish using ultra high performance liquid chromatography coupled with tandem mass spectrometry. J. Chromatogr. A 1218, 3511-3520.

Kim, J., Kang, J.H., Park, H., Baek, S.Y., Kim, Y.H., Chang, Y.S., 2012. Assessment of polybrominated diphenyl ethers (PBDEs) in serum from the Korean general population. Environ. Pollut. 164, 46-52.

Kim, J.W., Isobe, T., Sudaryanto, A., Malarvannan, G., Chang, K.H., Muto, M., Prudente, M., Tanabe, S., 2013. Organophosphorus flame retardants in house dust from the Philippines: Occurrence and assessment of human exposure. Environ. Sci. Pollut. R. 20, 812-822.

Kuo, Y.-Y., Zhang, H., Gerecke, A.C., Wang, J., 2014. Chemical Composition of Nanoparticles Released from Thermal Cutting of Polystyrene Foams and the Associated Isomerization of Hexabromocyclododecane (HBCD) Diastereomers. Aerosol Air Qual. Res. 14, 1114-1120.

Lankova, D., Svarcova, A., Kalachova, K., Lacina, O., Pulkrabova, J., Hajslova, J., 2015. Multi-analyte method for the analysis of various organohalogen compounds in house dust. Anal. Chim. Acta 854, 61-69.

Liagkouridis, I., Cousins, I.T., Cousins, A.P., 2014. Emissions and fate of brominated flame retardants in the indoor environment: A critical review of modelling approaches. Sci. Total. Environ. 491–492, 87-99.

Mizouchi, S., Ichiba, M., Takigami, H., Kajiwara, N., Takamuku, T., Miyajima, T., Kodama, H., Someya, T., Ueno, D., 2015. Exposure assessment of organophosphorus and organobromine flame retardants via indoor dust from elementary schools and domestic houses. Chemosphere 123, 17-25.

Newton, S., Sellström, U., de Wit, C.A., 2015. Emerging Flame Retardants, PBDEs, and HBCDDs in indoor and outdoor media in Stockholm, Sweden. Environ. Sci. Technol. 49, 2912-2920.

Roosens, L., Abdallah, M.A.-E., Harrad, S., Neels, H., Covaci, A., 2010. Current Exposure to Persistent Polychlorinated Biphenyls (PCBs) and Dichlorodiphenyldichloroethylene (p,p'-DDE) of Belgian Students from Food and Dust. Environ. Sci. Technol. 44, 2870-2875.

Roosens, L., Abdallah, M.A.E., Harrad, S., Neels, H., Covaci, A., 2009. Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: Preliminary results. Environ. Health Persp. 117, 1707-1712.

Sahlström, L.M.O., Sellström, U., de Wit, C.A., Lignell, S., Darnerud, P.O., 2015. Estimated intakes of brominated flame retardants via diet and dust compared to internal concentrations in a Swedish mother–toddler cohort. Int. J. Hyg. Environ. Health 218, 422-432.

Schreder, E.D., La Guardia, M.J., 2014. Flame Retardant Transfers from U.S. Households (Dust and Laundry Wastewater) to the Aquatic Environment. Environ. Sci. Technol. 48, 11575-11583.

Sjödin, A., Päpke, O., McGahee, E., Focant, J.-F., Jones, R.S., Pless-Mulloli, T., Toms, L.-M.L., Herrmann, T., Müller, J., Needham, L.L., Patterson Jr, D.G., 2008. Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries. Chemosphere 73, S131-S136.

Tan, J., Cheng, S.M., Loganath, A., Chong, Y.S., Obbard, J.P., 2007a. Polybrominated diphenyl ethers in house dust in Singapore. Chemosphere 66, 985-992.

Tan, J., Cheng, S.M., Loganath, A., Chong, Y.S., Obbard, J.P., 2007b. Selected organochlorine pesticide and polychlorinated biphenyl residues in house dust in Singapore. Chemosphere 68, 1675-1682.

UNEP, 2014. Report of the Persistent Organic Pollutants Review Committee on the work of its tenth meeting - Risk profile on decabromodiphenyl ether (commercial mixture, c decaBDE). Persistent Organic Pollutants Review Committee pp. 4-32.

Van den Eede, N., Dirtu, A.C., Ali, N., Neels, H., Covaci, A., 2012. Multi-residue method for the determination of brominated and organophosphate flame retardants in indoor dust. Talanta 89, 292-300.

Van den Eede, N., Dirtu, A.C., Neels, H., Covaci, A., 2011. Analytical developments and preliminary assessment of human exposure to organophosphate flame retardants from indoor dust. Environ. Int. 37, 454-461.

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119-1153.

Webster, T.F., Stapleton, H.M., 2012. Flame Retardants: Polybrominated Diphenyl Ethers and their Replacements. Dioxins and Health. John Wiley & Sons, Inc., pp. 89-108.

Whitehead, T.P., Brown, F.R., Metayer, C., Park, J.-S., Does, M., Petreas, M.X., Buffler, P.A., Rappaport, S.M., 2013. Polybrominated diphenyl ethers in residential dust: Sources of variability. Environ. Int. 57–58, 11-24.

Wilford, B.H., Shoeib, M., Harner, T., Zhu, J., Jones, K.C., 2005. Polybrominated diphenyl ethers in indoor dust in Ottawa, Canada: Implications for sources and exposure. Environ. Sci. Technol. 39, 7027-7035.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., La Guardia, M., McClean, M.D., Webster, T.F., 2007. Human Exposure to PBDEs: Associations of PBDE Body Burdens with Food Consumption and House Dust Concentrations. Environ. Sci. Technol. 41, 1584-1589.

3.3. Asthma and exposure to brominated flame retardants, polychlorinated biphenyls and organochlorine pesticides via house dust

Abstract

Brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCs) are major indoor contaminants associated with a large number of harmful effects in human health, including asthma. Exposure to these contaminants occurs largely via the ingestion of dust. Here we report the levels of BFRs, PCBs and OCs in dust samples collected from houses of asthmatics and non-asthmatic volunteers living in Covilhã, Portugal. No significant differences were found between the contaminants' levels in dust samples between the two groups (asthmatics and non-asthmatics houses). BFRs were the dominant group with polybrominated diphenyl ethers (PBDEs) and decabromodiphenyl ethane (DBDPE) exhibiting the highest levels, followed by hexabromocyclododecanes (HBCDDs), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs). Estimated daily intakes (EDIs) of BFRs, PCBs and OCs via the ingestion of dust were much lower than the established reference doses (RfD) and therefore the risk associated with dust ingestion is low.

Keywords: asthma, house dust, BFRs, PCBs, OCs.

3.3.1 Introduction

Asthma affects up to 334 million people worldwide (Global Asthma Network, 2014) and in Portugal it is prevalent in about 10% of the all population (de Araújo, 2016). According to the European Respiratory Society (2013), approximately one third of the population will develop asthma during their lifetime. This disease affects mostly children and elderly, though, the burden of asthma, measured by DALYs (Disability-adjusted life years), is greatest from the sixth decade of life onwards (Global Asthma Network, 2014). The older adults and elderly exhibit greater susceptibility for more exacerbations and related complications (de Araújo, 2016). This tendency might be related to the increased presence of comorbidities in these patients leading to a persistent decline in lung function (Reed, 2006).

Over the last decades the role of environmental factors in the etiology of asthma has received increasing attention, particularly in what concerns indoor contaminants. Mold and dampness, for example, are recognized as triggers for asthma, increasing the risk of asthma-related problems by 30-50% (European Respiratory Society, 2013). Besides these biological contaminants, several chemicals, including some indoor pollutants, have also been recognized as possible risk factors for the development of asthma (Hulin et al., 2012). However, a limited number of studies examined the associations between specific classes of indoor contaminants such as persistent organic pollutants (POPs) or flame retardants (FRs) and asthma (e.g. Hernandez et al., 2011; Araki et al., 2014; Canbaz et al., 2016; Meng et al., 2016a).

Brominated flame retardants (BFRs) are one of the most important groups of indoor contaminants that comprises several classes of flame retardants such as polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD) and emerging BFRs (e.g. 2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) and decabromodiphenyl ethane (DBDPE)) (Coelho et al., 2014; Coelho et al., 2016). Other well-known indoor contaminants are the polychlorinated biphenyls (PCBs) and the organochlorine pesticides (OCs) (e.g. hexachlorobenzene (HCB), chlordanes (CHLs), dichlorodiphenyltrichloroethanes (DDTs)). These indoor contaminants have been associated with many adverse health effects, such as endocrine and thyroid disruption, reduced IQ and neurodevelopmental delays in children (Dodson et al., 2012; Babrauskas et al., 2014), neurobehavioral disorders, interference with

the immune system functions (Hertz-Picciotto et al., 2008; Wang et al., 2015), cancer (IARC, 2015) and respiratory problems, including asthma (e.g. Hernandez et al., 2011; Meng et al., 2016a). As a consequence of their harmful effects the use of the majority of these compounds has already been banned in the European Union. The PCBs, HCB, CHLs, DDTs were discontinued since the 70's and 80's (EFSA, 2006b, a, 2007, 2010) whilst the PBDEs and HBCDDs were phased out in 2004 (penta- and octa- BDEs), 2008 (deca-BDEs) and 2015 (HBCDDs) (Coelho et al., 2014). BTBPE and DBDPE are currently used as replacements for octa-BDEs and deca-BDEs, respectively (Covaci et al., 2011). However, their physicochemical properties are similar to the replaced BFRs, and therefore they appear to have similar toxicological behaviour (Babrauskas et al., 2014).

Despite the restrictions on the manufacture and use of PCBs, OCs and BFRs, these contaminants are continuously released from the large range of goods in which they have been incorporated, being therefore spread into the environment and detected in a large number of media including air, soil, food and indoor dust. PCBs, HCB, CHLs and DDTs are in the list of the twelve contaminants initially recognized as POPs by the Global Stockholm Convention (Stockholm Convention, 2013), to which the commercial penta- and octa-BDEs and the HBCDDs were added latter.

Among all the possible sources of exposure to these contaminants, indoor dust is described as one of the most important (Mercier et al., 2011). In fact, indoor dust works as a reservoir of BFRs, PCBs and OCs and is widely used as a marker to evaluate the indoor contamination.

Considering that no data on the levels of BFRs, PCBs and OCs exists for the households of *Beira Interior* region in Portugal and considering the emerging evidences of the associations between the exposure to these contaminants in the indoor environment and respiratory diseases we launched a study at the University of Beira Interior in order to understand the role of the exposure to indoor contaminants and asthma.

In the present study we report the preliminary results on the levels of the selected BFRs, PCBs and OCs in house dust samples from adult asthmatics patients and non-asthmatics and the respective daily intakes via ingestion of dust.

3.3.2. Material and Methods

3.3.2.1. Samples collection

Participants were recruited, by convenience, at the *Centro Hospitalar Cova da Beira,* (Covilhã, Portugal) between September and November 2014, after the study approval by the Ethics Committee of the Faculty of Health Sciences, University of Beira Interior (CE-FCS-2012-034). Ten patients with asthma and four healthy controls were enrolled after signing an informed consent.

Recruited participants were asked to provide the bags used in their own domestic vacuum cleaners and to answer a questionnaire about housing characteristics. Details on the collected samples are described in Table 3.3.1. At the laboratory, the vacuum cleaner bags were opened to remove the house dust, and in order to obtain homogeneous samples each sample was sieved (< 500 µm, stainless steel). The samples were kept in amber glass vials and stored at -20^{0}C until chemical analysis.

3.3.2.2. Chemical analysis

About 1.5 g of each dust sample was extracted with androgynous sodium sulfate and a solution of acetone and hexane (1:1 *v/v*) through a high speed solvent extractor (SE-100, Mitsubishi Chemical Analytech). From the extract sample, an amount corresponding to 1 g dust was spiked with 5 ng of $^{13}C_{12}$-labeled PBDEs, $^{13}C_{12}$-labeled PCBs and $^{13}C_{12}$-labeled OCs and 10 ng of $^{13}C_{12}$-labeled HBCDDs as surrogates. This portion was then treated with hexane-washed sulphuric acid (98%), and passed through a multi-layer silica gel column for clean-up. The obtained extract was subjected to gel permeation chromatography (GPC: Bio-Beads S-X3, Bio-Rad, CA, 2 cm i.d.×50 cm) and eluted with a dichloromethane/hexane (1:1*v/v*) solution. The last fraction (containing the organohalogens) was concentrated using a rotatory evaporator and passed through 4 g of activated silica gel (Wakogel DX) packed in a glass column. The first fraction, eluted with 80 ml of dichloromethane/hexane (5% *v/v*), contained PBDEs, BTBPE, DBDPE, PCBs and OCs, while the second fraction, eluted with 100 ml of dichloromethane/hexane (25% *v/v*), contained HBCDDs. Both fractions were evaporated, and in order to ensure the surrogates'

recoveries the first fraction was spiked with $^{13}C_{12}$-labeled BDE 126 and BDE 205, $^{13}C_{12}$-labeled BDE 139 and pentachloro anisole while the second fraction was spiked with HBCDD-d_{18} (α-, β-, γ-HBCDD-d_{18}). PBDEs, BTBPE, DBDPE and PCBs were quantified using a gas chromatograph Agilent (7890 A) coupled with a mass spectrometer (Agilent 5975 C) and the silica column (J&W Scientific) DB-1MS (length: 30 m x internal diameter: 0.25 mm x film thickness: 0.25 µm) for PCBs and DB-5HT (15m x 0.25mm x 0.1µm) for the other analytes. For OCs, a GC (Agilent 7890A) coupled with a triple quadrupole mass spectrometer (MS/MS: Agilent 7000) and a HP-5MS column (30 m x 0.25 mm x 0.25 µm) was used.

Considering HBCDDs, the isomers were quantified with an Acquity UPLC (Waters, Tokyo, Japan) ultra-performance liquid chromatography equipped with Quattro Micro API triple–quadrupole mass spectrometer (Waters/Micromass, Tokyo, Japan) (LC-MS/MS) and the column Agilent Extend C18 (2.1 mm x 100 mm, 1.8 µm).

For quality assurance and control, procedural blanks were analysed simultaneously with every batch of samples. The limits of detection (LODs) were calculated as three times the standard deviation of background peaks in the procedural blanks. Concentrations below the limit of detection (LOD) were treated as zero for data analysis.

3.3.2.3. Statistical analysis

The software IBM SPSS Statistics 20 was used to evaluate the differences between groups (Mann-Whitney U test) and associations between the selected housing characteristics (information obtained from the questionnaires, Table 3.3.1) and contaminants' concentrations (Spearman rank correlation). Statistical analyses were carried out for a significance level of 0.05. The box-plot graphs (Figure 3.3.1) were obtained using Sigma Plot V11.0

3.3.2.4. Calculation of daily intakes

Participants' exposure to BFRs, PCBs and OCs through the ingestion of house dust was evaluated by calculating the estimated daily intakes (EDIs) based on the average house dust ingestion rate of 30 mg day^{-1} (US-EPA, 2011) and the highest dust ingestion of 100

mg day^{-1} (which corresponds to the worst case scenario) (Wilford et al., 2005) and the body weights of the participants (information gathered from the questionnaires).

3.3.3. Results and discussion

3.3.3.1. Housing characteristics

Eleven women and three men with ages varying from 31 to 75 years old participated in this study. Their body weights ranged between 51 and 112 kg. The analysed dust samples were collected from their houses wherein, 10 samples were from asthmatics participants (9 women and 1 men) and 4 from non-asthmatics (2 women and 2 men). The general characteristics of the houses are shown in Table 3.3.1.

Table 3.3.1. Housing characteristics.

Characteristics		**Asthmatics** (n=10)	**Non – asthmatics** (n=4)
Construction year [a]	1950 - 1969	2	1
	1970 - 1989	4	1
	1990 - 2010	3	2
House area (m^2) [b]	<100	1	1
	100 - 149	4	1
	≥ 150	1	2
House Type	Apartment	7	2
	Private House	3	2
Location	Urban	7	4
	Rural	3	-
Number of windows	<5	2	-
	5 - 9	7	1
	≥10	1	3
Reconstructions since 2000 [c]	Yes	3	3
	No	7	1

[a] Information not available for 1 house of the asthmatics group.
[b] Information not available for 4 houses of the asthmatics group.
[c] Including painting, floor and wall covering change, WC and kitchen reconstruction.

3.3.3.2. Concentrations in house dust

The contaminants levels distribution is shown in Figure 3.3.1.

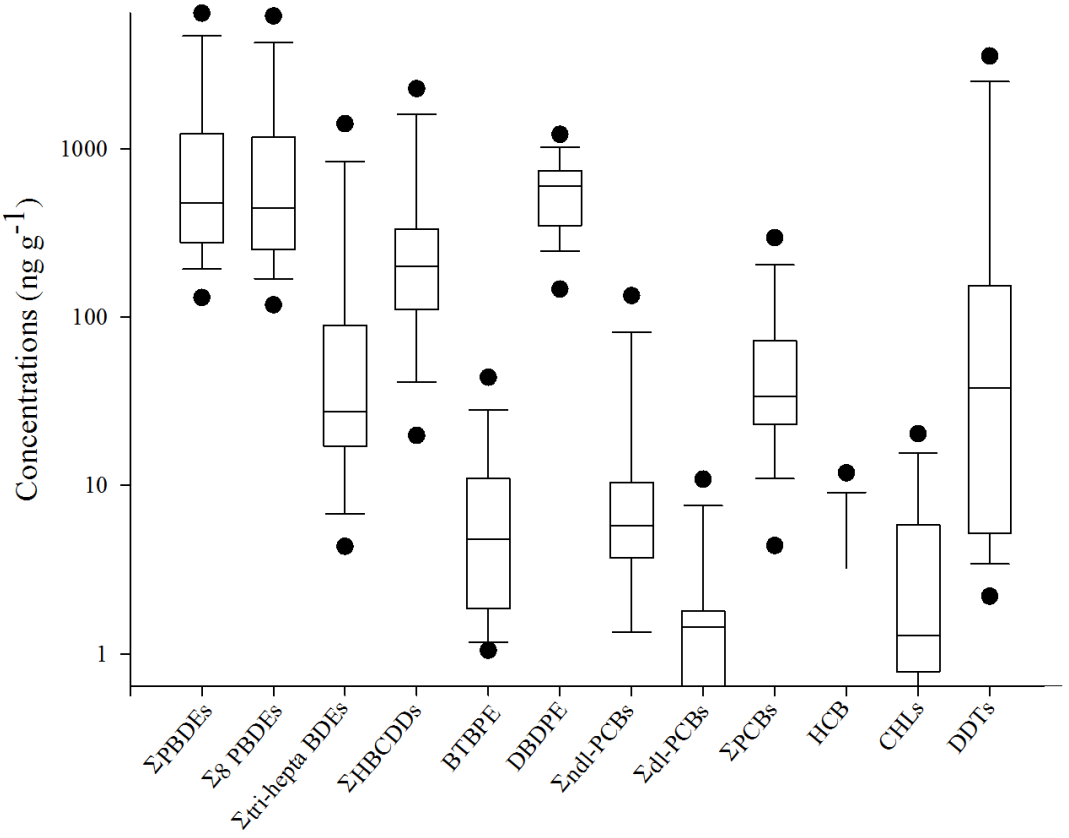

Figure 3.3.1. Boxplot summarizing the variation in total concentrations of the target compounds measured in the 14 house dust samples from Covilhã, Portugal. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).

3.3.3.2.1. BFRs

Figure 3.3.1 and Table 3.3.2 depicts the concentrations of the selected contaminants detected in house dust. For PBDEs only the data from the eight congeners of primary interest (BDE 28, 47, 99, 100, 153, 154, 183 and 209) (EFSA, 2011) were analysed individually. These congeners were detected in all the houses with the exception of BDE 28 and BDE 154 that were not detected in 30% and 10% of the samples from asthmatic patients' houses. The sums of their concentrations varied from 120 to 6200 ng g^{-1} (median: 730 ng g^{-1}) for asthmatics participants and from 260 to 1100 ng g^{-1} (median: 290 ng g^{-1}) for

the non-asthmatics. Their contribution for the total levels was over 85%. The concentrations of ∑ PBDEs ranged from 130 to 6400 ng g^{-1} (median: 850 ng g^{-1}) for asthmatics houses and from 280 to 1200 ng g^{-1} (median: 330 ng g^{-1}) for the non-asthmatics ones.

BDE 209 was the major congener in both groups, accounting approximately with 69% (asthmatics) and 82% (non-asthmatics) for the total ∑ PBDEs levels. This predominance of BDE 209 is in accordance with previous studies (Coelho et al., 2014; Fromme et al., 2015), especially the ones conducted in Europe, including three studies that also analysed house dust samples from Portugal (Fabrellas et al., 2005; Cunha et al., 2010; Coelho et al., 2016). Fromme et al. (2015) described that the contribution of BDE 209 to the ∑ PBDEs levels varied from 47 to 95%, considering several surveys from North America, Europe, Asia and Australia. This congener is the primary constituent of the deca-BDE commercial mixture most commonly used. This formulation was already restricted/banned (Coelho et al., 2014), however BDE 209 strongly associates with particles and therefore it concentrates in dust (Viganò et al., 2011), and consequently it becomes one of the principal PBDEs in house dust. For the asthmatics houses, the main congeners that followed the BDE 209 were: BDE 99, 47 and 153 (medians concentrations in Table 3.3.2), while for the non-asthmatics they were BDE 183, 99 and 47. The prevalence of these congeners reflects the likewise use of the penta- and octa-BDE commercial formulations in which they are predominant.

Concerning HBCDDs, the levels ranged between 20 and 940 ng g^{-1} (median: 210 ng g^{-1}) for asthmatics and from 62 to 2300 ng g^{-1} (median: 200 ng g^{-1}) for non-asthmatics and the three isomers were detected in all samples (Table 3.3.2). Considering the median values, α-HBCDD was the predominant isomer in both groups, contributing for over 55% to the ∑ HBCDDs, followed by γ-HBCDD and β-HBCDD. This distinct contribution for the total concentrations has been discussed in several studies. Although the amount of γ-HBCDD is about 75 to 89% in the commercial product (with α-HBCDD and β-HBCDD contributing with 10 – 13% and 1 – 12%, respectively), when considering the concentrations of each isomer in the environment, the importance of the α-HBCDD increases while the percentage of γ-HBCDD decreases with time (Koch et al., 2015). This can be explained with the thermal rearrangement of the HBCDD diastereomers: γ-HBCDD can isomerize to α-

HBCDD (more stable and less reactive) when the materials are treated at temperatures above 100ºC (Heeb et al., 2008; Kajiwara et al., 2009).

The emerging BFRs (EBFRs) were also detected in all samples (Table 3.3.2), wherein the DBDPE stands out with concentrations between 150 and 1200 ng g^{-1} (median: 480 ng g^{-1}) for asthmatics and from 460 to 830 ng g^{-1} (median: 720 ng g^{-1}) and in non-asthmatics houses. Overall, DBDPE medians were two orders of magnitude higher than the median levels of BTBPE (3.7 (asthmatics) and 9.2 (non-asthmatics) ng g^{-1}). In many cases, the concentrations of BTBPE were similar to those described for other house dust samples from other countries, while for DBDPE our levels were higher, when compared for example with studies conducted in Belgium (Ali et al., 2011), Norway (Cequier et al., 2014), Germany (Fromme et al., 2014), Sweden (Newton et al., 2015), New Zealand and Pakistan (Ali et al., 2012a; Ali et al., 2012b). However, in house dust samples from the United States (US) (Dodson et al., 2012; Brown et al., 2014; Schreder and La Guardia, 2014) and from Iraq (Al-Omran and Harrad, 2016), higher levels of BTBPE were obtained, though the levels of DBDPE were also lower. For this EBFRs, higher levels were detected in the US (mean: 1030 ng g^{-1}) (La Guardia and Hale, 2015) and Sweden (median: 1700 ng g^{-1}) (Sahlström et al., 2012).

3.3.3.2.2. PCBs

The indicator non-dioxin like PCBs (ndl-PCBs) and the dioxin-like PCBs (dl-PCBs) levels are shown in Table 3.3.2. PCBs were mainly detected at concentrations lower than BFRs, probably because, in Portugal, their industrial and commercial use was phased out by 1976 and totally banned in 1988 (Garcia et al., 2015) while the use of BFRs was only regulated from the 2000s onwards as previously mentioned. Their total levels varied from 4.4 – 110 ng g^{-1} (median: 32 ng g^{-1}) for asthmatics and from 25 – 300 ng g^{-1} (median: 49 ng g^{-1}) for non-asthmatics.

Ndl-PCBs were the predominant congeners being detected in all samples from the non-asthmatic group and almost all samples from the asthmatic group. In fact, for the asthmatic group PCB 52, 138 and 153 were not detected in 10% of the dust samples whilst PCB 28 was not detected in 30% of the houses. No congener of the ndl-PCBs stands out since their median levels are similar (about 1 ng g^{-1}, in both groups), with the exception of PCB 28

that registered the lowest medians (0.45 and 0.48 ng g^{-1}). Although with similar median levels, one of the houses from the non-asthmatic group (built in 1955) stands out with the highest concentrations of ndl-PCBs in dust, particularly for PCB 101, 138, 153 and 180 (see in Table 3.3.2). The levels of these penta, hexa and hepta congeners in this dust sample might reflect a source in the house of the commercial mixtures Aroclor 1254 and 1260 that were widely used in Europe, mainly in the production of paints and sealants (Cachada et al., 2009).

Considering the dl-PCBs, seven congeners (77, 81, 114, 123, 126, 157, and 169) were not detected (levels below LOD). For the remaining congeners (PCB 105, 118, 156, 167, 189), PCB 118, 105 and 156 exhibited the highest detection frequencies in both groups, 90 – 100%, 70 – 75% and 60 – 75%, respectively (asthmatics – non-asthmatics). PCB 118 was the major contributor for the sum of the concentrations of dl-PCBs. Such as for the ndl-PCBs, the same house constructed in 1955, exhibited the highest levels of PCB 118, 156 and 167 (highest concentrations in the ranges in Table 3.3.2).

3.3.3.2.3. OCs

Considering the OCs (Table 3.3.2), CHLs and DDTs were detected in all samples, and the detection frequencies of HBC were 50 and 75% (for asthmatics and non-asthmatics, respectively). DDTs were the most prevalent compounds in both groups with median concentrations of 47 and 25 ng g^{-1} (asthmatics and non-asthmatic, respectively). These concentrations were about 2 or 3 orders of magnitude higher than HCB (medians: 0.36 and 0.3 ng g^{-1}) and CHL (medians: 0.88 and 2 ng g^{-1}). The predominance of DDTs is in accordance with other studies worldwide that showed higher levels of DDTs than HCB and CHLs in house dust samples (Tan et al., 2007b; Dirtu and Covaci, 2010; Brauner et al., 2011; Ali et al., 2013; Wang et al., 2013). Thereby, DDT was ubiquitous in the evaluated houses which reinforces the fact that house dust is an important source of DDTs, despite the restrictions in their use in the early 1970´s and their ban as pesticides since 1986 in the European Union (EFSA, 2006a).

Table 3.3.2. BFRs, PCBs and OCs concentrations (ng g^{-1}) measured in the 14 dust samples with the indication of detection frequency (DF %), mean, median and range. <LOD: bellow limit of detection.

	Non-asthmatics (n =4)				Asthmatics (n = 10)			
	DF %	**Mean**	**Median**	**Range**	**DF %**	**Mean**	**Median**	**Range**
BFRs								
BDE 28	100	0.23	0.2	0.17 – 0.36	70	0.53	0.27	< LOD – 1.7
BDE 47	100	6.3	4.2	2 – 15	100	44	10	1.7 – 310
BDE 99	100	6.3	4.5	3.9 – 12	100	110	21	1.5 – 740
BDE 100	100	1.3	0.97	0.59 – 2.6	100	19	2.9	0.27 – 140
BDE 153	100	2.1	1.8	1.3 – 3.5	100	17	3.5	0.22 – 110
BDE 154	100	0.59	0.54	0.44 – 0.83	90	13	1.7	< LOD – 96
BDE 183	100	7	6.2	1.4 – 14	100	5.3	3.1	0.56 – 21
BDE 209	100	460	270	230 – 1100	100	1100	590	110 – 6000
Σ PBDEs[a]	_	520	330	280 – 1200	_	1500	850	130 – 6400
Σ tri-hepta-BDE[b]	_	24	23	14 – 33	_	200	43	4.3 – 1400
Σ 8 PBDEs[c]	_	490	290	260 – 1100	_	1300	730	120 – 6200
α-HBCDD	100	220	140	36 – 570	100	180	120	12 – 710
β-HBCDD	100	42	19	6.3 - 120	100	35	21	3.1 – 150
γ-HBCDD	100	420	39	20 – 1600	100	56	56	5.1 – 120
Σ HBCDDs	_	680	200	62 – 2300	_	270	210	20 - 940
BTBPE	100	16	9.2	1.9 – 44	100	5.2	3.7	1 – 12
DBDPE	100	680	720	460 – 830	100	540	480	150 – 1200
PCBs								
Non-dioxin-like PCBs								
PCB 28	100	0.63	0.48	0.3 – 1.2	70	0.97	0.45	< LOD – 4.8
PCB 52	100	1.1	1.1	0.36 – 1.9	90	1.7	0.99	< LOD – 9.8
PCB 101	100	5.7	1.2	0.3 – 20	100	1.4	1	0.31 – 5.2
PCB 138	100	12	1.3	0.4 – 44	90	1.8	1.6	< LOD – 3.7
PCB 153	100	11	1.2	0.44 – 42	90	1.6	1.4	< LOD – 3.2
PCB 180	100	6.9	0.65	0.27 – 26	100	1	1	0.29 – 2.3

	Non-asthmatics (n =4)				Asthmatics (n = 10)			
	DF %	**Mean**	**Median**	**Range**	**DF %**	**Mean**	**Median**	**Range**
Dioxin-like PCBs*								
PCB 105	75	0.44	0.36	< LOD – 1.1	70	0.35	0.37	< LOD – 1
PCB 118	100	1.8	0.65	0.2 – 5.6	90	0.89	0.82	< LOD – 2.9
PCB 156	75	0.67	0.12	< LOD – 2.4	60	0.11	0.12	< LOD – 0.26
PCB 167	50	0.34	0.041	< LOD – 1.3	40	0.046	< LOD	< LOD – 0.13
PCB 189	25	0.11	< LOD	< LOD – 0.46	0	< LOD	< LOD	< LOD
Σ ndl-PCBs[d]	_	37	6.3	2.1 – 130	_	8.4	5.8	0.6 – 29
Σ dl-PCBs[e]	_	3.4	1.2	0.2 – 11	_	1.4	1.4	< LOD – 4.3
Σ 18 PCBs[f]	_	41	7.5	2.3 – 150	_	9.8	7	0.6 – 33
ΣPCBs	_	100	49	25 – 300	_	46	32	4.4 – 110
OCs								
HCB	75	0.29	0.36	< LOD – 0.43	50	2.6	0.3	< LOD – 12
CHLs[g]	100	3.3	0.88	0.64 – 11	100	4.2	2	0.4 – 20
DDTs[h]	100	910	25	4.6 – 3600	100	210	47	2.2 – 1500

[a] Sum of all congeners; [b] Sum of BDE 28, 47, 99, 100, 153, 153 and 183; [c] Sum of BDE 28, 47, 99, 100, 153, 153, 183 and 209.
[d] Sum of non-dioxin-like PCBs (28, 52, 101, 138, 153 and 180); [e] dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189); [f] sum of dioxin-like PCBs and non-dioxin-like PCBs. [g] Sum of trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor and oxychlordane; [h] Sum of o,p-DDT, o,p-DDD, o,p-DDE, p,p'-DDT, p,p'-DDD, p,p'-DDE.
*PCB 77, 81, 114, 123, 126, 157, 169 were not included since they were not detected (< LOD) in all samples.

3.3.3.3. Concentrations of the selected compounds in house dust and differences between groups

The concentrations of PBDEs, HBCDDs, BTBPE, DBDPE, PCBs and OCs were compared between both groups, non-asthmatics and asthmatics houses, and no statistically significant differences were observed for any compound (Mann Whitney U test, $p > 0.05$). However, the small sample size may have compromised the occurrence of statistically significant differences.

Overall, PBDEs and DBDPE exhibited the highest median concentrations in both groups, with higher median levels of PBDEs in dust samples from asthmatics houses and with higher median levels of DBDPE in the house dust samples from the non-asthmatics (Table 3.3.2). The number of studies assessing the levels of these compounds in house dust, between non - asthmatics and asthmatics patients, is very limited, especially for adults. Canbaz et al. (2016) evaluated the association between the levels of PBDEs found in indoor dust (collected from mattresses) and the development of childhood asthma, and found no statistically significant differences between both analysed groups (non-asthmatic and asthmatic children). In turn, Meng et al. (2016b), found statistically significant differences between the levels of few PBDEs, PCBs and OCs in house dust from non-asthmatics and asthmatic children, with higher levels of BDE 47, 99 and 209, and PCB 8 and 49 in dust from the asthmatic children' houses and higher levels of OCs in non-asthmatics group.

Although there are some studies reporting few positive correlations between the pre-natal or young children exposure to these contaminants (especially PBDEs, PCBs and DDTs) and the development or aggravation of asthma (see for e.g. Mamane et al., 2015; Meng et al., 2016a), there is still no evidence in the association between this exposure and the risk of asthma-related symptoms (Gascon et al., 2013). Therefore, in order to better describe this possible association, as well as to evaluate the main exposure sources to these indoor contaminants, more studies are necessary. Such studies should focus not only in children, but also in adults, as the onset of asthma during childhood is associated to a major risk of persistency in the adult age.

3.3.3.4. Association with housing characteristics

Since there were no statistically significant differences between the concentrations of the target contaminants from house dust samples provided by asthmatics and non-asthmatics participants, the associations between the contaminants' levels and the housing characteristics are discussed as a single group.

Regarding the PBDEs, HBCDDs, BTBPE and DBDPE no statistically significant correlations were found between their levels and all the considered housing characteristics (construction year, house area, house type, location, number of windows and reconstructions), with the exception of BDE-28 which levels were significantly lower in houses with more windows (Spearman Correlation, r= 0.594, df= 12, p= 0.049) (Figure C1, Annex C).

The levels of BFRs in house dust may be influenced by several factors, including construction and also reconstruction year. In fact, some studies reported that the levels of PBDEs in house dust samples are more influenced by the year of the last reconstruction rather than the year of construction Kalachova et al. (2012). Depending on the construction/reconstruction decade the levels and the type of BFRs may vary. For example, during the late 1990s and early 2000s PBDEs were probably more frequently used. This is particularly relevant for deca-BDE that accounted for about 80% of the global PBDE demand in 1999 and 2001 (EFSA, 2011). Secondly, from the time when their use started to be restricted, the use of BTBPE and DBDPE, as alternatives for octa-BDEs and deca-BDEs, increased. A recent study conducted in the United Kingdom, Kuang et al. (2016) demonstrated that the levels of BDE 209 were significantly lower in house dust samples collected in 2015 compared to those collected in 2006 and 2007, while the levels of DBDPE were significantly higher in the samples from 2015. However, and similarly to the present study where no correlations between the levels of BFRs in dust and the house characteristics (including construction/reconstruction years), other studies could not find any trend in BFRs levels and housing characteristics (e.g. Tan et al., 2007a; Wu et al., 2007; Cunha et al., 2010; Coelho et al., 2016).

Regarding the PCBs, it was possible to observe a trend associated with the age of the house. PCB 105 (Spearman Correlation, r= 0.707, df= 11, p= 0.005), PCB 118 (Spearman Correlation, r= 0.603, df= 11, p= 0.029) and Σ dl-PCBs (Spearman Correlation, r= 0.633,

df= 11, p= 0.021) were significantly higher in older houses (Figures C2 – C4, Annex C). This association between the levels of dl-PCBs in house dust and the houses' age, was also observed in house dust samples from Australia (Hinwood et al., 2014). Such results might indicate the decrease in the use of PCBs in Portugal due to the legislation implemented since 1976. Furthermore, statistically significant differences were observed between the levels of Σ PCBs in houses from different locations (Urban versus rural; Mann Whitney U test, H= 30.000, P= 0.038) and in different types of houses (apartments versus private houses; Mann Whitney U test, H= 7.000, P= 0.042). The Σ PCBs concentrations tended to be higher in houses located in urban locations and in apartments (Figures C5 and C6, Annex C).

For OCs, it would be expected to find higher levels in older houses (due to the restrictions in their use implemented in the 1970's (see sections 3.3.1 and 3.3.3.2.3), however no correlations were found between their levels in house dust and any of the houses characteristics.

3.3.3.5. Estimated daily intakes

The exposure to the target compounds present in dust may occur via inhalation, ingestion and dermal contact. Although the relative contribution of each exposure pathway to the total intakes may vary between the chemical class (Whitehead et al., 2015), the ingestion of dust stands out as the major route (Dirtu and Covaci, 2010; Besis et al., 2015; Meng et al., 2016b). Therefore, the participants' exposure to BFRs, PCBs and OCs via dust ingestion was evaluated by calculating the estimated daily intakes (EDIs) based on the average and the highest dust ingestion rates (30 and 100 mg day^{-1}) and the body weights of each participant (see section 2.4). EDIs are shown in Table 3.3.3, as well as the defined reference dose (RfD) values.

Regarding the worst case scenario (dust ingestion rate of 100 mg day^{-1}), the medians of the EDIs (asthmatics– non-asthmatics) of BFRs were 1.1 – 0.45, 0.25 – 0.31, 4.7 x 10^{-3} – 0.015and 0.56 – 0.74 ng kg-bw^{-1} day^{-1} for ∑ PBDEs, ∑ HBCDDs, BTBPE and DBDPE, respectively. These EDIs disclose a higher intake of PBDEs by the asthmatics group and higher intake of DBDPE by the non-asthmatics. However, in both groups, EDIs were more than 1000 times lower than the established RfD, 7000 ng kg-bw^{-1} day^{-1} for BDE 209

(considering that BDE 209 is the dominant congener, section 3.3.3.2.1), 200 x 10^3 ng kg-bw^{-1} day^{-1} for HBCDDs, 243 x 10^3 ng kg-bw^{-1} day^{-1} for BTBPE and 333 x 10^3 ng kg-bw^{-1} day^{-1} for DBDPE (Van den Eede et al., 2011; Ali et al., 2012b).

For PCBs, and considering the worst case scenario, the calculated EDIs of the ∑ PCBs (medians: 0.051 and 0.056 ng kg-bw^{-1} day^{-1}, for asthmatics and non-asthmatics) were much lower than the RfD of 1000 ng kg-bw^{-1} day^{-1} (Ali et al., 2012b). The obtained EDIs, disclose that, the participants' intake through house dust ingestion of BFRs and PCBs is very low.

For the OCs, in the worst case scenario, the medians of the EDIs (asthmatics – non-asthmatics) were 5.8 x 10^{-4} – 4.6 x 10^{-4} ng kg-bw^{-1} day^{-1} for HCB, 2.8 x 10^{-3} – 1.2 x 10^{-3} ng kg-bw^{-1} day^{-1} for CHLs and 0.064 – 0.042 ng kg-bw^{-1} day^{-1} for DDTs. As expected from the obtained DDTs levels registered in the house dust samples, the intakes of DDTs through the ingestion of house dust were greater in both groups than the other two OCs. Nevertheless, the EDIs were more than 3 orders of magnitude lower than the RfD of 800 ng kg-bw^{-1} day^{-1} set for HCB (US-EPA, 1991) and 500 ng kg-bw^{-1} day^{-1} for CHLs (US-EPA, 1998) and for DDTs (US-EPA, 1988).

Overall, BFRs exhibited the highest EDIs, when compared with PCBs and OCs. Such higher EDIs for BFRs might be a consequence of the latter introduction of restrictions in their use (2000s-10s versus 1970s-80s) (see section 3.3.1). For all the target compounds, the EDIs for asthmatics and non-asthmatics were far below the established RfD, though they were detected in the majority of the analysed samples.

3.3.4. Conclusions

The present study disclosed for the first time the widespread presence of BFRs, PCBs and OCs in house dust samples from Beira Interior region in Portugal. These contaminants were present both in dust samples from the houses of asthmatics and non-asthmatics participants. There were no statistically significant differences between both groups (asthmatics and non-asthmatics), possibly due to the small sample size.

As expected, BFRs exhibited the highest levels in house dust, wherein the DBDPE stands out in the non-asthmatics houses while the PBDEs showed higher concentrations in the asthmatic houses.

Table 3.3.3. Estimated daily intakes of BFRs, PCBs and OCs via dust ingestion, calculated from the house dust concentrations and considering the average house dust ingestion rate of 30 mg day^{-1} and the highest dust ingestion of 100 mg day^{-1} and the body weights of the participants.

		Non-asthmatics (n=4)			Asthmatics (n=10)			
	Dust Intake *mg day^{-1}*	**Mean** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Median** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Mean** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Median** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**RfD** *ng kg-bw^{-1} day^{-1}*
Σ PBDEs[a]	30	0.21 (16)	0.14 (9.9)	0.083 – 0.5 (8.4 – 35)	0.62 (44)	0.33 (25)	0.068 – 2.7 (3.9 – 190)	BDE 47: 100 BDE 99: 100 BDE 153: 200 BDE 209: 7000[g]
	100	0.71 (52)	0.45 (33)	0.28 – 1.7 (28 – 120)	2.1 (150)	1.1 (85)	0.23 – 8.9 (13 – 640)	
Σ tri-hepta-BDE[b]	30	0.009 (0.71)	0.009 (0.7)	0.005 – 0.014 (0.45 – 1)	0.082 (6.1)	0.016 (1.3)	0.002 – 0.56 (0.13 – 42)	
	100	0.03 (2.4)	0.028 (2.3)	0.017 – 0.047 (1.5 – 3.3)	0.27 (20)	0.054 (4.3)	0.007 – 1.9 (0.43 – 140)	
Σ 8 PBDEs[c]	30	0.2 (15)	0.12 (8.8)	0.073 – 0.47 (7.8 – 33)	0.56 (40)	0.29 (22)	0.061 – 2.6 (3.6 – 190)	
	100	0.66 (49)	0.41 (29)	0.24 – 1.6 (26 – 110)	1.9 (130)	0.96 (73)	0.2 – 8.6 (11 – 620)	
Σ HBCDDs	30	0.2 (21)	0.094 (5.9)	0.02 – 0.61 (1.9 – 68)	0.11 (8.2)	0.074 (6.2)	0.01- 0.37 (0.6 – 28)	200 000[h]
	100	0.68 (68)	0.31 (20)	0.066 – 2 (6.2 – 230)	0.38 (27)	0.25 (21)	0.034 – 1.2 (2 – 94)	
BTBPE	30	0.0053 (0.48)	0.0044 (0.28)	0.0006 – 0.012 (0.056 – 1.3)	0.0022 (0.15)	0.0014 (0.11)	0.0005 – 0.05 (0.031 – 0.37)	243 000[g]
	100	0.018 (1.6)	0.015 (0.92)	0.002 – 0.039 (0.19 – 4.4)	0.0073 (0.52)	0.0047 (0.37)	0.0017 – 0.017 (0.1 – 1.2)	
DBDPE	30	0.26 (20)	0.22 (22)	0.20 – 0.38 (14 – 25)	0.24 (16)	0.17 (15)	0.076 – 0.72 (4.4 – 37)	333 333[g]
	100	0.85 (68)	0.74 (72)	0.65 – 1.3 (46 – 83)	0.79 (54)	0.56 (48)	0.25 – 2.4 (15 – 120)	
ΣPCBs	30	0.042 (3.1)	0.017 (1.5)	0.0091 – 0.13 (0.74 – 8.9)	0.019 (1.4)	0.015 (0.97)	0.0017 – 0.047 (0.13 – 3.4)	1000[g]
	100	0.14 (10)	0.056 (4.9)	0.03 – 0.42 (2.5 – 30)	0.064 (4.6)	0.051 (3.2)	0.0058 – 0.16 (0.44 – 11)	

		Non-asthmatics (n=4)			Asthmatics (n=10)			
	Dust Intake *mg day^{-1}*	**Mean** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Median** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Mean** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Median** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**Range** *ng kg-bw^{-1} day^{-1} (ng day^{-1})*	**RfD** *ng kg-bw^{-1} day^{-1}*
Σ ndl-PCBs[d]	30	0.016 (1.1)	0.0029 (0.19)	0.00056 – 0.058 (0.062 – 4)	0.0035 (0.25)	0.0026 (0.17)	0.00024 – 0.012 (0.018 – 0.87)	
	100	0.053 (3.7)	0.0096 (0.63)	0.0019 – 0.19 (0.21 – 13)	0.012 (0.84)	0.0088 (0.58)	0.00079 – 0.039 (0.06 – 2.9)	
Σ dl-PCBs[e]	30	0.0014 (0.1)	0.00053 (0.035)	0.00005 – 0.0047 (0.006 – 0.33)	0.00059 (0.042)	0.00056 (0.043)	0 – 0.0017 (0 – 0.13)	
	100	0.0048 (0.34)	0.0018 (0.12)	0.00018 – 0.016 (0.02 – 1.1)	0.002 (0.14)	0.0019 (0.14)	0 – 0.0058 (0 – 0.43)	
Σ 18 PCBs[f]	30	0.017 (1.2)	0.0034 (0.22)	0.00061 – 0.062 (0.068 – 4.4)	0.0041 (0.3)	0.0033 (0.21)	0.00024 – 0.013 (0.018 – 1)	
	100	0.058 (4.1)	0.011 (0.75)	0.002 – 0.21 (0.23 – 15)	0.014 (0.98)	0.011 (0.7)	0.00079 – 0.045 (0.06 – 3.3)	
HCB	30	0.00012 (0.0086)	0.00014 (0.011)	0 – 0.00019 (0 – 0.013)	0.0011 (0.079)	0.00018 (0.0089)	0 – 0.0047 (0 – 0.36)	800[i]
	100	0.00039 (0.029)	0.00046 (0.036)	0 – 0.00063 (0 – 0.043)	0.0036 (0.26)	0.00058 (0.03)	0 – 0.016 (0 – 1.2)	
CHLs	30	0.0014 (0.1)	0.00036 (0.026)	0.00017 – 0.0047 (0.019 – 0.33)	0.0018 (0.13)	0.00085 (0.059)	0.00013 – 0.0081 (0.012 – 0.61)	500[j]
	100	0.0046 (0.33)	0.0012 (0.088)	0.00057 – 0.016 (0.064 – 1.1)	0.0059 (0.42)	0.0028 (0.2)	0.00045 – 0.027 (0.04 – 2)	
DDTs	30	0.39 (27)	0.012 (0.75)	0.0012 – 1.5 (0.14 – 110)	0.09 (6.2)	0.019 (1.4)	0.00087 – 0.62 (0.066 – 44)	500[k]
	100	1.3 (91)	0.042 (2.5)	0.0041 – 5.1 (0.46 – 360)	0.3 (21)	0.064 (4.7)	0.0029 – 2.1 (0.22 – 150)	

[a] Sum of all congeners; [b] Sum of BDE 28, 47, 99, 100, 153, 153 and 183; [c] Sum of BDE 28, 47, 99, 100, 153, 153, 183 and 209.
[d] Sum of non-dioxin-like PCBs (28, 52, 101, 138, 153 and 180); [e] dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189); [f] sum of dioxin-like PCBs and non-dioxin-like PCBs.
[g] Ali et al. (2012b); [h] Van den Eede et al. (2011); [i] US-EPA (1991); [j] US-EPA (1998); [K] US-EPA (1988).

Regarding the PCBs, ndl-PCBs were the main contributors to the ∑ PCBs concentrations. DDTs were the major pesticides detected in the surveyed houses.

Generally, no significant correlations were found between the levels of the studied contaminants in house dust and the housing characteristics, with the exception of (i) PCB 105, PCB 118 and ∑ dl-PCBs levels that were significantly higher in older houses; (ii) ∑ PCBs, concentrations that tended to be higher in houses located in urban locations and in apartments and (iii) BDE 28 that disclosed lower concentrations in houses with more windows.

Participants' daily intake of PBDES, HBCDDs, BTBPE, DBDPE, PCBs, BHC, CHLs and DDTs via dust ingestion were far below the established RfD, demonstrating that for the studied population the exposure to these contaminants through the ingestion of dust is low. Although the EDIs are not of concern, these compounds were detected in a considerable number of samples, showing high detection frequencies (100% in most cases) which demonstrates their ubiquitous presence. It is important to highlight that there is a large number of sources to these compounds and that their intake may occur through different pathways, thereby the discussed levels are only part of the real exposure.

Acknowledgements

The authors wish to acknowledge the volunteers who participated in this study. The assistance of R. Okubo and S. Nishioka during chemical analysis is deeply acknowledged as well as the assistance provided by Dr. N. M. Tue during data analysis. This work was supported by European Funds through COMPETE and by National Funds through the Portuguese Science Foundation (FCT) within projects PEst-OE/SAU/UI0709/2014. Ana C. A. Sousa and Sónia D. Coelho acknowledge FCT for the grants SFRH/BPD/65884/2009 and SFRH/BD/78168/2011 (supported by funding from the Human Potential Operational Programme POPH, inscribed in the National Strategic Reference Framework and partially subsidized by the European Social Fund). This study was partly supported by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) to a project on Joint Usage/Research Center – Leading Academia in Marine and Environmental Research (LaMer), Ehime University. Further support was provided by Grants-in-Aid for Scientific Research (S: 26220103, A: 25257403, A: 16H01784, B: 25281050) of the Japanese Ministry of Education, Science, Sports, Culture and Technology (MEXT) and Japan Society for the Promotion of Science (JSPS).

Supplementary data

Supplementary data to this subchapter (Figures C1 – C6) can be found in Annex C.

References

Al-Omran, L.S., Harrad, S., 2016. Polybrominated diphenyl ethers and "novel" brominated flame retardants in floor and elevated surface house dust from Iraq: Implications for human exposure assessment. Emerg. Contam. 2, 7-13.

Ali, N., Ali, L., Mehdi, T., Dirtu, A.C., Al-Shammari, F., Neels, H., Covaci, A., 2013. Levels and profiles of organochlorines and flame retardants in car and house dust from Kuwait and Pakistan: Implication for human exposure via dust ingestion. Environ. Int. 55, 62-70.

Ali, N., Dirtu, A.C., Eede, N.V.D., Goosey, E., Harrad, S., Neels, H., t Mannetje, A., Coakley, J., Douwes, J., Covaci, A., 2012a. Occurrence of alternative flame retardants in indoor dust from New Zealand: Indoor sources and human exposure assessment. Chemosphere 88, 1276-1282.

Ali, N., Harrad, S., Goosey, E., Neels, H., Covaci, A., 2011. "Novel" brominated flame retardants in Belgian and UK indoor dust: Implications for human exposure. Chemosphere 83, 1360-1365.

Ali, N., Van den Eede, N., Dirtu, A.C., Neels, H., Covaci, A., 2012b. Assessment of human exposure to indoor organic contaminants via dust ingestion in Pakistan. Indoor Air 22, 200-211.

Araki, A., Saito, I., Kanazawa, A., Morimoto, K., Nakayama, K., Shibata, E., Tanaka, M., Takigawa, T., Yoshimura, T., Chikara, H., Saijo, Y., Kishi, R., 2014. Phosphorus flame retardants in indoor dust and their relation to asthma and allergies of inhabitants. Indoor Air 24, 3-15.

Babrauskas, V., Fuoco, R., Blum, A., 2014. Chapter 3 - Flame Retardant Additives in Polymers: When do the Fire Safety Benefits Outweigh the Toxicity Risks? . in: Kiliaris, P. (Ed.). Polymer Green Flame Retardants. Elsevier, Amsterdam, pp. 87-118.

Besis, A., Botsaropoulou, E., Voutsa, D., Samara, C., 2015. Particle-size distribution of polybrominated diphenyl ethers (PBDEs) in the urban agglomeration of Thessaloniki, northern Greece. Atmos. Environ. 104, 176-185.

Brauner, E.V., Mayer, P., Gunnarsen, L., Vorkamp, K., Raaschou-Nielsen, O., 2011. Occurrence of organochlorine pesticides in indoor dust. J. Environ. Monit. 13, 522-526.

Brown, F.R., Whitehead, T.P., Park, J.S., Metayer, C., Petreas, M.X., 2014. Levels of non-polybrominated diphenyl ether brominated flame retardants in residential house dust samples and fire station dust samples in California. Environ. Res. 135, 9-14.

Cachada, A., Lopes, L.V., Hursthouse, A.S., Biasioli, M., Grčman, H., Otabbong, E., Davidson, C.M., Duarte, A.C., 2009. The variability of polychlorinated biphenyls levels in urban soils from five European cities. Environ. Pollut. 157, 511-518.

Canbaz, D., van Velzen, M.J., Hallner, E., Zwinderman, A.H., Wickman, M., Leonards, P.E., van Ree, R., van Rijt, L.S., 2016. Exposure to organophosphate and polybrominated diphenyl ether flame retardants via indoor dust and childhood asthma. Indoor Air 26, 403-413.

Cequier, E., Ionas, A.C., Covaci, A., Marcé, R.M., Becher, G., Thomsen, C., 2014. Occurrence of a Broad Range of Legacy and Emerging Flame Retardants in Indoor Environments in Norway. Environ. Sci. Technol. 48, 6827-6835.

Coelho, S.D., Sousa, A.C.A., Isobe, T., Kim, J.-W., Kunisue, T., Nogueira, A.J.A., Tanabe, S., 2016. Brominated, chlorinated and phosphate organic contaminants in house dust from Portugal. Sci. Total. Environ. 569–570, 442-449.

Coelho, S.D., Sousa, A.C.A., Isobe, T., Tanabe, S., Nogueira, A.J.A., 2014. Flame retardants in indoor dust - A review on the levels of polybrominated diphenyl ethers and hexabromocyclododecanes. Curr. Org. Chem. 18, 2218-2230.

Covaci, A., Harrad, S., Abdallah, M.A.E., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532-556.

Cunha, S.C., Kalachova, K., Pulkrabova, J., Fernandes, J.O., Oliveira, M.B.P.P., Alves, A., Hajslova, J., 2010. Polybrominated diphenyl ethers (PBDEs) contents in house and car dust of Portugal by pressurized liquid extraction (PLE) and gas chromatography-mass spectrometry (GC-MS). Chemosphere 78, 1263-1271.

de Araújo, A.T., 2016. 11º Relatório - Previnir as doenças respiratórias, acompanhar e reabilitar os doentes. Observatório Nacional das Doenças Respiratórias, pp. 261.

Dirtu, A.C., Covaci, A., 2010. Estimation of Daily Intake of Organohalogenated Contaminants from Food Consumption and Indoor Dust Ingestion in Romania. Environ. Sci. Technol. 44, 6297-6304.

Dodson, R.E., Perovich, L.J., Covaci, A., Van Den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., Rudel, R.A., 2012. After the PBDE phase-out: A broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol. 46, 13056-13066.

EFSA, 2006a. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to DDT as an undesirable substance in animal feed. EFSA J. 1 - 69.

EFSA, 2006b. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to Hexachlorobenzene as undesirable substance in animal feed. EFSA J. 1 - 49.

EFSA, 2007. Chlordane as undesirable substance in animal feed (1) Scientific Panel on Contaminants in the Food Chain EFSA J. 1-53.

EFSA, 2010. Results of the monitoring of non dioxin-like PCBs in food and feed. EFSA J. 35.

EFSA, 2011. Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA J. 274.

European Respiratory Society, 2013. European Lung White Book. in: Gibson, J., Loddenkemper, R., Sibille, Y., Lundbäck, B. (Eds.).

Fabrellas, B., Martinez, A., Ramos, B., Ruiz, M.L., Navarro, I., de la Torre, A., 2005. Results of an european survey based on PBDEs analysis in household dust. Dioxin. Organohalogen Compounds, Toronto, Canada, pp. 452–454.

Fromme, H., Becher, G., Hilger, B., Völkel, W., 2015. Brominated flame retardants – Exposure and risk assessment for the general population. Int. J. Hyg. Environ. Health 219, 1-23.

Fromme, H., Hilger, B., Kopp, E., Miserok, M., Völkel, W., 2014. Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and "novel" brominated flame retardants in house dust in Germany. Environ. Int. 64, 61-68.

Garcia, J.S., Pimpão, A., Baltazar, L., Vilão, R., 2015. Inventário Nacional de PCB - Ano de 2014 Agência Portuguesa do Ambiente, I.P. Departamento de Resíduos / Divisão de Responsabilidade Ambiental e Solos Contaminados, pp. 3-23.

Gascon, M., Morales, E., Sunyer, J., Vrijheid, M., 2013. Effects of persistent organic pollutants on the developing respiratory and immune systems: A systematic review. Environ. Int. 52, 51-65.

Global Asthma Network, 2014. The Global Asthma Report 2014. Auckland, New Zealand: Global Asthma Network, pp. 94.

Heeb, N.V., Bernd Schweizer, W., Mattrel, P., Haag, R., Gerecke, A.C., Schmid, P., Zennegg, M., Vonmont, H., 2008. Regio- and stereoselective isomerization of hexabromocyclododecanes (HBCDs): Kinetics and mechanism of γ- to α-HBCD isomerization. Chemosphere 73, 1201-1210.

Hernandez, A.F., Parron, T., Alarcon, R., 2011. Pesticides and asthma. Curr. Opin. Allergy Clin. Immunol. 11, 90-96.

Hertz-Picciotto, I., Park, H.-Y., Dostal, M., Kocan, A., Trnovec, T., Sram, R., 2008. Prenatal Exposures to Persistent and Non-Persistent Organic Compounds and Effects on Immune System Development. Basic Clin. Pharmacol. Toxicol. 102, 146-154.

Hinwood, A.L., Callan, A.C., Heyworth, J., Rogic, D., de Araujo, J., Crough, R., Mamahit, G., Piro, N., Yates, A., Stevenson, G., Odland, J.O., 2014. Polychlorinated biphenyl (PCB) and dioxin concentrations in residential dust of pregnant women. Environ. Sci. Process. Impacts 16, 2758-2763.

Hulin, M., Simoni, M., Viegi, G., Annesi-Maesano, I., 2012. Respiratory health and indoor air pollutants based on quantitative exposure assessments. Eur. Respir. J. 40, 1033-1045.

IARC, 2015. Polychlorinated Biphenyls and Polybrominated Biphenyls. IARC monographs on the evaluation of carcinogenic risks to humans, pp. 513.

Kajiwara, N., Sueoka, M., Ohiwa, T., Takigami, H., 2009. Determination of flame-retardant hexabromocyclododecane diastereomers in textiles. Chemosphere 74, 1485-1489.

Kalachova, K., Hradkova, P., Lankova, D., Hajslova, J., Pulkrabova, J., 2012. Occurrence of brominated flame retardants in household and car dust from the Czech Republic. Sci. Total. Environ. 441, 182-193.

Koch, C., Schmidt-Kötters, T., Rupp, R., Sures, B., 2015. Review of hexabromocyclododecane (HBCD) with a focus on legislation and recent publications concerning toxicokinetics and -dynamics. Environ. Pollut. 199, 26-34.

Kuang, J., Ma, Y., Harrad, S., 2016. Concentrations of "legacy" and novel brominated flame retardants in matched samples of UK kitchen and living room/bedroom dust. Chemosphere 149, 224-230.

La Guardia, M.J., Hale, R.C., 2015. Halogenated flame-retardant concentrations in settled dust, respirable and inhalable particulates and polyurethane foam at gymnastic training facilities and residences. Environ. Int. 79, 106-114.

Mamane, A., Raherison, C., Tessier, J.-F., Baldi, I., Bouvier, G., 2015. Environmental exposure to pesticides and respiratory health. Eur. Respir. Rev. 24, 462-473.

Meng, G., Feng, Y., Nie, Z., Wu, X., Wei, H., Wu, S., Yin, Y., Wang, Y., 2016a. Internal exposure levels of typical POPs and their associations with childhood asthma in Shanghai, China. Environ. Res. 146, 125-135.

Meng, G., Nie, Z., Feng, Y., Wu, X., Yin, Y., Wang, Y., 2016b. Typical halogenated persistent organic pollutants in indoor dust and the associations with childhood asthma in Shanghai, China. Environ. Pollut. 211, 389-398.

Mercier, F., Glorennec, P., Thomas, O., Bot, B.L., 2011. Organic contamination of settled house dust, a review for exposure assessment purposes. Environ. Sci. Technol. 45, 6716-6727.

Newton, S., Sellström, U., de Wit, C.A., 2015. Emerging Flame Retardants, PBDEs, and HBCDDs in indoor and outdoor media in Stockholm, Sweden. Environ. Sci. Technol. 49, 2912-2920.

Reed, C.E., 2006. The natural history of asthma. J. Allergy Clin. Immun. 118, 543-548; quiz 549-550.

Sahlström, L., Sellström, U., De Wit, C.A., 2012. Clean-up method for determination of established and emerging brominated flame retardants in dust. Anal. Bioanal. Chem. 404, 459-466.

Schreder, E.D., La Guardia, M.J., 2014. Flame Retardant Transfers from U.S. Households (Dust and Laundry Wastewater) to the Aquatic Environment. Environ. Sci. Technol. 48, 11575-11583.

Stockholm Convention, 2013. Listing of POPs in the Stockholm Convention. http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx. (accessed August, 2015).

Tan, J., Cheng, S.M., Loganath, A., Chong, Y.S., Obbard, J.P., 2007a. Polybrominated diphenyl ethers in house dust in Singapore. Chemosphere 66, 985-992.

Tan, J., Cheng, S.M., Loganath, A., Chong, Y.S., Obbard, J.P., 2007b. Selected organochlorine pesticide and polychlorinated biphenyl residues in house dust in Singapore. Chemosphere 68, 1675-1682.

US-EPA, 1988. p,p'-Dichlorodiphenyltrichloroethane (DDT); CASRN 50-29-3. Integrated Risk Information System. https://www.epa.gov/iris. (accessed April, 2016).

US-EPA, 1991. Hexachlorobenzene; CASRN 118-74-1. Integrated Risk Information System. https://www.epa.gov/iris. (accessed April, 2016).

US-EPA, 1998. Chlordane (Technical); CASRN 12789-03-6. Integrated Risk Information System. https://www.epa.gov/iris. (accessed April, 2016).

US-EPA, 2011. Exposure Factors Handbook 2011 Edition (Final). U.S. Environmental Protection Agency, Washington, DC.

Van den Eede, N., Dirtu, A.C., Neels, H., Covaci, A., 2011. Analytical developments and preliminary assessment of human exposure to organophosphate flame retardants from indoor dust. Environ. Int. 37, 454-461.

Viganò, L., Roscioli, C., Guzzella, L., 2011. Decabromodiphenyl ether (BDE-209) enters the food web of the River Po and is metabolically debrominated in resident cyprinid fishes. Sci. Total. Environ. 409, 4966-4972.

Wang, B.-L., Pang, S.-T., Sun, J.-P., Zhang, X.-L., Li, X.-L., Sun, Y.-G., Lu, X.-M., Zhang, Q., 2015. Levels of polychlorinated biphenyls in settled house dust from urban dwellings in China and their neurodevelopmental effects on preschool-aged children. Sci. Total. Environ. 505, 402-408.

Wang, W., Huang, M.-J., Wu, F.-Y., Kang, Y., Wang, H.-S., Cheung, K.C., Wong, M.H., 2013. Risk assessment of bioaccessible organochlorine pesticides exposure via indoor and outdoor dust. Atmos. Environ. 77, 525-533.

Whitehead, T.P., Crispo Smith, S., Park, J.-S., Petreas, M.X., Rappaport, S.M., Metayer, C., 2015. Concentrations of persistent organic pollutants in California women's serum and residential dust. Environ. Res. 136, 57-66.

Wilford, B.H., Shoeib, M., Harner, T., Zhu, J., Jones, K.C., 2005. Polybrominated Diphenyl Ethers in Indoor Dust in Ottawa, Canada: Implications for Sources and Exposure. Environ. Sci. Technol. 39, 7027-7035.

Wu, N., Herrmann, T., Paepke, O., Tickner, J., Hale, R., Harvey, E., La Guardia, M., McClean, M.D., Webster, T.F., 2007. Human Exposure to PBDEs: Associations of PBDE Body Burdens with Food Consumption and House Dust Concentrations. Environ. Sci. Technol. 41, 1584-1589.

CHAPTER 4

GENERAL CONCLUSION AND FINAL REMARKS

Chapter 4. General conclusion and final remarks

The present work evaluates the human exposure to several organic and inorganic contaminants in Portugal, by characterizing two important pathways of exposure, the ingestion of food (Chapter 2) and the ingestion of house dust (Chapter 3). The characterization of dietary intake was performed by evaluating the levels of BFRs (PBDEs, HBCDDs, BTBPE and DBDPE), PCBs, OCs (HCHs, HCB, CHLs and DDTs) lead and cadmium in duplicate diet samples collected over a period of seven days by volunteers from the University of Aveiro academic community. The characterization of the ingestion through house dust was performed in two distinct studies. In one study the levels of PFRs (TCEP, TDCIPP, TPP, TNBP, EHDPP, TMPP, TPEP, TPHP and TEHP), BFRs (PBDEs, HBCDDs, BTBPE and DBDPE) and PCBs were assessed in house dust samples from Aveiro and Coimbra. The participants were recruited by convenience and provided the vacuum cleaners bags used to clean their houses and from where the dust samples were collected. In the other study, a similar collection of dust was conducted but in houses from asthmatics and non-asthmatics volunteers living in Covilhã, and the analysed compounds were BFRs (PBDEs, HBCDDs, BTBPE and DBDPE), PCBs and OCs (HCB, CHLs and DDTs).

The results obtained under the framework of the present thesis disclose that diet and house dust are important sources of the target compounds and that both pathways contributed to the studied Portuguese populations' exposure.

The diet study (Chapter 2) revealed that duplicate diet samples exhibited low levels of the analysed POPs (PBDEs, HBCDDs, PCBs and OCs) and therefore the estimated dietary intakes of the participants were low and below the established guidance values for human risk assessment (RfDs and TDIs). Therefore, the health risk associated with the consumption of food is low for this academic community. Although the detected concentrations were negligible, PBDEs, HBCDDs, PCBs and OCs were present in the analysed diet samples despite the legislative measures to reduce their use (see subchapters 3.1, 3.2 and 3.3). DDTs and HCHs were even detected in 100% of the diet samples, even though they were banned in Europe since the 1970s. These results confirm their persistency and bioaccumulation, furthermore, since these contaminants are lipophilic they

will be concentrated in the human fat tissue, leading to possible long-term adverse consequences.

Regarding Pb and Cd, both toxic metals were detected in 100% of the analysed duplicate diet samples. These frequencies of detection were expected, since, as previously mentioned, foodstuffs are major sources of Cd and Pb. The exposure of the participants (adults from the same academic community) to Pb was even associated to an increased risk for elevated systolic blood pressure (3.3%) and nephrotoxic effects (26.7%). For Cd, 35% of the participant premenopausal women exhibited EWIs higher than the set TWI, suggesting increased health risks for these women, mainly renal tubular effects (subchapter 2.4).

Overall, the results obtained in Chapter 2 showed that the participants were exposed to the target compounds (BFRs, PCBs, OCs, Pb and Cd) via the consumption of food, confirming the importance of this route of exposure to environmental contaminants in Portugal, similarly to what was previously demonstrated in other countries (see subchapters 2.2, 2.3 and 2.4).

Considering the exposure through house dust, our results disclosed that all the groups of analysed compounds (BFRs, PFRs, PCBs and OCs) were detected, being therefore, indicators of indoor contamination.

In the 28 house dust samples collected in Aveiro and Coimbra, PFRs generally exhibited higher concentrations and were the major group of contaminants followed by BFRs, where PBDEs (mainly BDE 209) and HBCDDs stand out, followed by DBDPE and then by the PCBs group. These results might reflect the increased consumption of PFRs as alternatives for other FRs (as described in Chapter 1). For the 14 house dust samples from Covilhã (asthma study; chapter 3.3), among the measured contaminants, BFRs were the dominant group, with higher levels of PBDEs (mainly BDE 209) and DBDPE in both groups (asthmatics and non-asthmatics), followed by HBCDDs, DDTs (major group of OCs) and PCBs. In both studies, even considering the worst case scenario of the daily ingestion of dust (100 mg day^{-1}), the participants' estimated intakes of the evaluated compounds (FRs, PCBs and OCs) were much lower than the set reference doses, however, the detection frequencies were high (above 50%) in most cases. Such results, demonstrates the widespread presence of the target compounds in Portuguese houses and confirms that the participants were exposed to those contaminants through the ingestion of house dust.

Considering both sources of exposure (diet and house dust) and analysing in more detail each group of contaminants it is possible to conclude that the main detected congeners/isomers of PBDEs, HCBCDDs and PCBs were the same in the three studies. In the case of PBDEs, BDE 209, 99 and 47 exhibited higher detection frequencies as well as higher levels; for the HBCDD isomers, α-HBCDD was the most abundant one followed by γ-HBCDD and β-HBCDD. As for PCBs, the major congeners were: PCB 153, 138 and 101. These coincident trends show that in Portugal both sources – food and house dust – disclose the same contamination scenario, which might reflect the commercial formulations most commonly used, as discussed in the previous chapters.

As shown in Figure 4.1, house dust arises as the major source of the surveyed POPs (PBDEs, HBCDDs, PCBs, HCHs, HCB, CHLs, DDTs) and the EBFRs (BTBPE and DBDPE, structurally similar to predecessors), especially for the flame retardants. This is due to the fact that settled dust acts as a repository and concentrator of indoor contaminants. However, the relative contribution of the two exposure pathways depends on the contaminants' ingestion rate, which will be influenced by the quantity of ingested food and dust (in the kg day^{-1} range (adults: 1.9 kg day^{-1}) for food, and in the mg day^{-1} range for house dust (adults highest ingestion: 100 mg day^{-1})). Hence, when it comes to the main route of exposure, the ingestion of food stands out as demonstrated by the estimated dietary daily intakes (Figure 4.2), particularly in the case of the PCBs and DDTs. These two group of contaminants exhibited the highest EDIs (Figure 4.2). Nevertheless, the median concentrations of these compounds detected in the duplicate diet samples were two orders of magnitude lower than the ones detected in the house dust samples from Covilhã (0.17 versus 34 ng g^{-1} for PCBs, and 0.38 versus 38 ng g^{-1} for DDTs, respectively) (Figure 4.1; see also, Tables 2.2.1 and 3.3.2).

Besides the traditional POPs, within the framework of this thesis it was also possible to analyse for the first time ever in Portugal the levels of PFRs in environmental samples, namely in house dust. Our results disclosed that the indoor environment is contaminated by this group of chemicals and that the concentrations of phosphorous flame retardants is higher than the traditional FRs, which might be a consequence of the search for alternatives to the banned BFRs (subchapter 3.2).

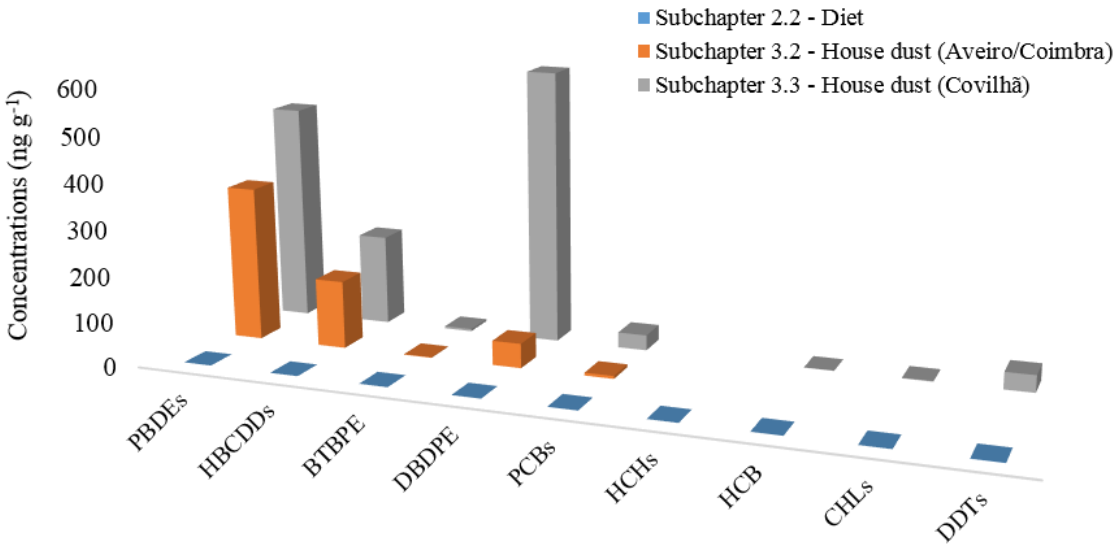

Figure 4.1. Median levels of the target compounds detected in the Portuguese studied samples (duplicate diet and house dust samples). See subchapters 2.2, 3.2 and 3.3.

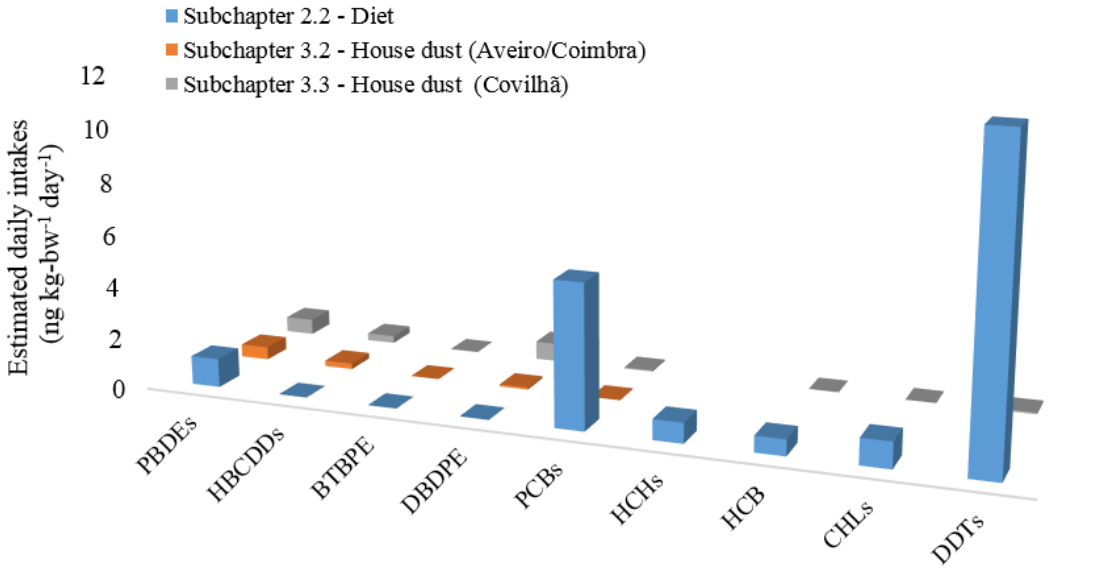

Figure 4.2. Median estimated daily intakes of the target compounds via the ingestion of food or house dust in Portugal. See subchapters 2.2, 3.2 and 3.3.

Overall, considering the organic contaminants (BFRs, EBFRs, PFRs, PCBs and OCs) our results disclose that despite the fact that POPs and EBFRs were detected both in diet and house dust samples, their levels were relatively low and the risk associated with the ingestion of these two matrices is low. In contrast, the results obtained for the ingestion of

lead and cadmium through diet, reveal a different scenario with high levels detected and consequently with estimated weekly intakes higher than the recommended ones.

Besides these major routes of human exposure, the intake of the target contaminants increases if all sources and pathways are considering, e.g. dermal contact and inhalation of dust and air and therefore the risks might be higher. Thus future monitoring surveys should take these factors also into account.

In order to assess the real exposure to the target compounds in Portugal, as well as their impacts in human health, such as developmental neurotoxicity, cardiovascular effects, nephrotoxicity, cancers and asthma (see subchapters 2.3, 2.4 and 3.3), further work should be done considering the several exposure routes and the contaminants' body burden. By assessing the levels of the studied compounds also in human matrixes such as blood, hair, nails and breast milk and associate them with increased health risks. These steps are important in order to accomplish the risk assessment of chemicals, described in Chapter 1.

In summary, the present thesis demonstrates that both diet and house dust are contaminated by the target contaminants. This is the first study monitoring the presence of flame retardants in the Portuguese diet as well as the majority of the studied POPs in indoor dust from Portuguese houses. Also for PCBs, OCs, Pb and Cd, this was the first study monitoring their levels in Portuguese duplicate diet samples. Therefore, this pioneer work constitutes an important baseline for future studies in Portugal and comes as an important complement for the global data on the occurrence and human exposure to these environmental contaminants.

Annexes

Annex A

Supplementary data from subchapter 3.1: **Flame retardants in indoor dust - a review on the levels of polybrominated diphenyl ethers and hexabromocyclododecanes.**

Table 1. Median and range concentrations (ng g^{-1}) of major polybrominated diphenyl ether congeners in indoor dust reported worldwide in the last 10 years. n: number of samples.

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[1]	Germany	40	Houses	0.6	0.2-4.4	17.1	3.3 - 1910	23.9	2.6 - 2850	4.2	0.5 - 314	6	0.4 - 420	2.7	0.3 - 210	6.1	0.9 - 464	265	18.6 - 19100
[2]	US	120	Houses			< 400	< 400 - 9860	304	< 400 - 22500	< 300	< 300 - 3400								
[3]	UK	10	Houses	0.35	0.05 - 33	24.8	10 - 1980	44	18 - 2100	8.5	3.9 - 230	23	< 0.1 - 170	4.7	2.1 - 110	9.5	< 0.1 - 87	7100	3800 - 19900
	Denmark	1	Houses	3[(a)]		66[(a)]		< 0.1[(a)]		11[(a)]		23[(a)]		1.8[(a)]		11[(a)]		260[(a)]	
	Finland	1	Houses	0.1[(a)]		9.9[(a)]		8.8[(a)]		3.5[(a)]		3.8[(a)]		0.8[(a)]		< 0.1[(a)]		100[(a)]	
[4]	Germany	1	Houses	< 0.3[(a)]		31[(a)]		37[(a)]		8[(a)]				4.5[(a)]		1.8[(a)]		2800[(a)]	

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
	Spain	4	Houses	< 0.3	< 0.1 - 0.7	13	11 - 16	17.5	14 - 21	3.7	2.4 - 4.2			0.8	1.5 - 3	4.1	1.7 - 39	425	92 - 1700
	France	8	Houses	0.25	0.1 - 4.8	24	7.3 - 260	28.5	7.5 - 720	6.2	1.4 - 92			2.8	1 - 64	8	2.6 - 44	420	69 - 3400
	Italy	1	Houses	< 0.3[(a)]		23[(a)]		36[(a)]		6.5[(a)]				6.5[(a)]		62[(a)]		1600[(a)]	
[5]	Belgium	23	Houses and offices			< 20	< 20 - 751	29	< 20 - 944	< 20	< 20 - 207	< 20	< 20 - 86	< 20	< 20 - 33	< 20	< 20 - 75	< 100	<100 - 303677
[6]	US	9	Houses	3	< 3 - 135	364	49 - 10538	612	79 - 13841	103	13 - 2605	61.2	7.5 - 1097	53.7	6.2 - 1094	18.6	3.4 - 36	665	536 - 65777
[7]	US	17	Houses	14.8[(d)]	2.9 - 76.5	644	103 - 7610	676	162 - 13800	119	25.9 - 2090	64.4	11.7 - 1510	72.8	11.8 - 1250	17.6	1.3 - 168	1350	162 - 8750
[8]	Canada	68	Houses	3	< LOD - 550	300	21 - 33000	430	19 - 60000	73	4.1 - 21000	49	3.1 - 25000	37	1.5 - 18000	19	< LOD - 650	630	74 - 10000
[9]	Kuwait	17	Houses	0.12	< LOD - 3.47	2.7	0.11 - 65	3.37	0.04 - 35.74	0.68	0.01 - 8.62	0.72	< LOD - 4.36	0.85	< LOD - 6.14	1.28	< LOD - 24.61	82.9	0.8 - 338.1

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[10]	Spain	4	Houses			22.4(e)	8.1 - 59.2	26.25 (e)	9.4 - 64.2	5.375 (e)	2.74 - 19.6	2.975 (e)	2.73 - 3.42	2.63(e)	1.63 - 4.65				
[11]	Japan	19	Houses	0.62	0.24 - 7.6	5.4	1.1 - 22	5.1	0.18 - 39	1.1	0.16 - 5.7	2.5	0.66 - 11	0.9	0.37 - 5.7	7.5	1.5 - 50	550	100 - 2600
[12]	UK	9	Houses	0.29	<0.1 - 0.29	16.3	4.2 - 62	35.8	8 - 85	5.7	1.2 - 16	6.5	1 - 9.7	4.0	1.4 - 33	8.9	0.88 - 20	3796	1401 - 54795
[13]	Sweden	5	Houses	0.71(e)	0.51 - 7.85	25.9(e)	12.6 - 160	57.6(e)	23.9 - 194	7.94(e)	< 2.21 - 92.3	4.7(e)	2.39 - 7.1	4.59(e)	2.41 - 4.93	2.32(e)	< 1.58 - 16.6	158(e)	43.9 - 1560
[14]	Spain	6	Houses			11.55 (e)	6.9 - 69.5	10.1(e)	6.23 - 60	2.06(e)	1 - 18.2	2.96(e)	0.98 - 9.07	3.24(e)	0.69 - 9.69	21.72 (e)	4.55 - 142	183.5 (e)	58.4 - 1615
[15]	Singapore	31	Houses	0.6	< LOD - 5.8	20	< LOD - 1500	24	< LOD - 6300	4.2	< LOD - 1200	6.9	< LOD - 1400	3.5	< LOD - 960	8.5	1.5 - 180	1000	68 - 13000
[16]	US	11	Houses			670	240 - 14610	1010	269 - 14800	170	< LOD - 2780	110	< LOD - 560	90	< LOD - 460			< LOD	< LOD - 9600

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[17]	Japan	13	Houses	0.8[e]	< 0.4 - 550	7[e]	< 0.6 - 2800	7[e]	< 4 - 1700	1.4[e]	< 1 - 260	2[e]	< 0.6 - 150	2[e]	< 0.6 - 84	3.1[e]	< 1 - 16	110[e]	14 - 3200
[18]	US	20	Houses	16.3[b, d]	1.6 - 120.5	1865[b]	44.5 - 16840	2460[b]	330.6 - 24510	436.3[b]	71 - 4274	234.4[b]	27.7 - 2377	182.8[b]	27.4 - 2061	27.9[b]	1.7 - 229.7	4502[b]	791.9 - 184600
	US	20	Houses	10.5[b, d]	0.7 - 119.4	837[b]	54.3 - 15000	1170[b]	56.5 - 22850	204[b]	11.4 - 3786	124.2[b]	4.2 - 2870	94.4[b]	4.3 - 2332	32.9[b]	0.1 - 1617	1703[b]	24 - 36130
	US	20	Houses	6.4[b,d]	1.6 - 58.6	337.6[b]	22.5 - 5596	536.4[b]	86.6 - 8847	76.9[b]	5.2 - 1475	47[b]	1.8 - 783.5	35[b]	0.5 - 626.4	15.1[b]	2.6 - 410.9	1811[b]	227.8 - 263000
[19]	Belgium	8	Houses															148.5[e]	8 - 307
	Romania	1	Houses															27[a]	
	Spain	1	Houses															140[a]	
[20]	UK	30	Houses	< LOD	< LOD - 2.1	10	1.2 - 58	20	2.8 - 180	3.4	< LOD - 17	5	< LOD - 110	2.8	< LOD - 16	4.2	< LOD - 550	8100	< LOD - 2200000

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[21]	New Zealand	20	Houses	0.65	0.11 - 2.1	24	3.3 - 150	51	6.4 - 380	8.9	1.2 - 70	5.4	0.66 - 35	5.1	0.56 - 35				
	UK	28	Houses	0.53	< LOD - 2.3	13	1.2 - 160	23	2.8 - 320	4.2	0.53 - 50	5.2	0.63 - 110	3.3	0.31 - 31	13	2 - 550	2800	120 - 520000
	Canada	10	Houses	4.1	1.4 - 20	140	47 - 720	330	80 - 1800	65	14 - 420	43	9.4 - 260	39	6.2 - 280	9	7 - 30	560	290 - 1100
	US	20	Houses	14	2.1 - 140	410	82 - 3300	820	150 - 6000	160	33 - 840	110	19 - 1800	89	19 - 2200	16	4 - 170	1300	530 - 3300
[22]	US	11	Houses			2920[e]	655 - 8830	4430[e]	807 - 11700	793[e]	165 - 2000								
[23]	Australia	10	Houses			60	20 - 1400	100	26 - 3400	18	5 - 550	13	5 - 510	9	< 2 - 300	14	< 6 - 99	730	23 - 13000
	Germany	10	Houses			< 14	< 14 - 22	10	< 4 - 28	< 6	< 6 - 7	< 6	< 6 - 22	< 6		< 6	< 6 - 120	63	< 6 - 410
	UK	10	Houses			22	7 - 180	28	10 - 300	4	< 2 - 52	5	< 2 - 53	3	< 2 - 25	5	< 3 - 18	10000	910 - 54000
	US	10	Houses			430	230 - 3000	880	70 - 3700	150	< 8 - 660	140	5 - 650	80	< 4 - 260	70	< 4 - 4000	2000	120 - 21000

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[24]	US	1	Houses	59[(a)]		1042[(a)]		747[(a)]		111[(a)]		42[(a)]		14[(a)]		4.6[(a)]		40[(a)]	
[25]	US	39	Houses			3750	112 - 107000	3830	102 - 170000	756	< 42 - 30900								
	US	10	Houses			1260	192 - 31100	1160	209 - 44900	223	44.5 - 8720								
[26]	US	12	Houses	26	max. 130	2000	max. 46000	4600	max. 79000	1200	max. 78000	110	max. 790	190	max. 1300	220	max. 7600	190	max. 66000
[27]	Germany	34	Houses	0.25	0.07–1.87	9.08	1.71 - 255	12.5	1.83 - 390	2.49	0.28 - 81.1	2.69	0.3 - 41.1	1.62	0.17 - 42.1	4.26	0.29 - 60.4	312	29.7 - 1460
[28]	China	46	Houses	1.04	0.28 - 17.86	8.42	2.59 - 149	9.49	1.25 - 303.5	1.26	0.14 - 60.88	4.2	1.4 - 118.1	1.87	0.45 - 39.68	8.46	2.81 - 47.42	2637	537.2 - 9602
[29]	US	38	Houses			520[(b)]		614[(b)]		120[(b)]		73[(b)]		51[(b)]				1398[(b)]	
[30]	US	12	Houses	< 0.1	< 0.1 - 1.3	40.1	16.7 - 354	95	25.6 - 664	16.1	0.75 - 122	25.9	< 0.04 - 81.5	9.6	2.48 - 56.9	10.5	10.5 - 64	903	327 - 9210

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[31]	US	24	Houses			500	max. 7620	838	max. 9220	180	max. 2830								
[32]	Thailand	53	Houses	0.1	< LOD - 0.55	1.9	0.12 - 59	3.4	0.19 - 138	0.72	< LOD - 21	0.91	< LOD - 17	0.48	< LOD-18				
[33]	Belgium	19	Houses															106	19.2 - 588
[34]	Japan	2	Houses	< LOD		2.45[(f)]	2.4 - 2.5	3[(f)]	2.4 - 3.2	0.54[(f)]	0.44 - 0.64	2.01[(f)]	0.42 - 3.6	0.99[(f)]	0.18 - 1.8			390[(f)]	160 - 620
[35]	Australia	5	Houses			18[(e)]	8 - 54	25[(e)]	11 - 82	5[(e)]	0.7 - 17	7[(e)]	3 - 14	3[(e)]	1 - 9	10[(e)]	3 - 28	151[(e)]	37 - 587
[36]	Australia	10	Houses			56.5[(e)]	24 - 434	87[(e)]	36 - 862	17.5[(e)]	10 - 155	7.4[(e)]	1 - 139			2.8[(e)]	< 0.002 - 948	291[(e)]	95 - 1585
[37]	US	4	Houses	5.15[(e)]	0.5 - 10.2	205.35[(e)]	22.3 - 617.2	244.8[(e)]	57.9 - 1278	44.15[(e)]	9 - 199.3	20.4[(e)]	4.3 - 141.4	16.35[(e)]	3.1 - 117.5	7.1[(e)]	4 - 27.6	1038[(e)]	734 - 4156
	US	1	Houses	10.4[(f)]		1450.5[(f)]		2015.5[(f)]		323.6[(f)]		109.3[(f)]		94.95[(f)]		5.7[(f)]		799.5[(f)]	

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[38]	Portugal	9	Houses	< LOQ[e]	< LOQ - 4	19[e]	< LOQ - 52	6[e]	3 - 32	2[e]	< LOQ - 7	3[e]	< LOQ - 8	< LOQ[e]	< LOQ - 3	4[e]	2 - 21	953[e]	148 - 1832
[39]	Belgium	45	Houses	0.4	max. > 0.9 (P95)	8.1	max. > 62.4 (P95)	8.9	max. > 110 (P95)	1.1	max. > 12.1 (P95)	2.2	max. > 43.9 (P95)	0.9	max. > 4.7 (P95)	1.4	max. > 9.5 (P95)	313	2.5 - 5295
[40]	China	76	Houses	0.87	0.12 - 68.75	7.58	1.44 - 236.86	5.63	1.02 - 303.51	0.67	0.12 - 60.88	3.56	0.74 - 118.10	1.36	0.25 - 39.68	6.06	1.54 - 47.42	1792	175.1 - 9602
[41]	US	50	Houses	11.7[d]	3.14 - 84	390	100 - 8627	427	79.3 - 12967	99.9	19 - 2164	55.9	13.2 - 1352	51.3	6.69 - 1093	17.4	3.76 - 688	1482	425 - 32366
[42]	Philippines	25	Houses	0.28	0.078 - 1.1	4	< LOD - 91	6.3	1.1 - 411	0.83	0.19 - 32	2.2	< LOD - 41	0.52	0.11 - 26	2.4	0.35 - 10	118	16 - 524
[43]	China	27	Houses	< 0.51 (Tri-BDEs)		< 10.4 (Tetra-BDEs)		< 14 (Penta-BDEs)		< 14 (Penta-BDEs)		< 4.53 (Hexa-BDEs)		< 4.53 (Hexa-BDEs)		< 8.4 (Hepta-BDEs)		4039	498 – 40500

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[44]	US	27	Houses	20	7 - 108	1308	335 - 6413	2561	532 - 14735	378	80 - 2929	326	64 - 2690	151	42 - 1580	26	5 - 2520	2476	582 - 721333
[45]	Germany/US	26	Houses															622	< 100 - 37900
[46]	China	23	Houses	37.6	6.77 - 122	102	26.7 - 2740	75.4	15 - 9447	84.9	22.1 - 221	10.9	< LOD - 649	8.36	< LOD - 714	77.7	14.5 - 797	975	346 - 15795
[47]	US	25	Houses			2780	301 - 126000	4450	613 - 220000	1050	141 - 41100								
	US	29	Houses			3100	185 - 29200	5840	367 - 43200	1060	84 - 7490								
[48]	Denmark	42	Houses	0.241 (d)	< LOQ - 5.55	16.9	3.29 - 962	13.6	2.72 - 1764	2.46	0.351 - 292	2.48	0.547 - 182	1.29	0.121 - 143	3.95	< LOQ - 47.3	332	55.7 - 580664
[49]	China	27	Houses			7.29	0.8 - 543	7.1	0.67 - 842	0.76	0.16 - 186	2.75	0.30 - 60.4	1.03	< LOQ - 47.7	8.4	< LOQ - 65.9	4040	498 - 405000

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
	China	10	Houses			1.21	< LOQ - 3.89	1.61	< LOQ - 5.09	< LOQ	< LOQ - 0.26	1.09	0.63 - 4.14	< LOQ	< LOQ - 1.78	2.3	< LOQ - 5.64	670	182 - 1680
[50]	New Zealand	34	Houses			25	< 2 - 100	30	3 - 220							2	< 2 - 240	435	< 5 - 6970
	New Zealand	16	Houses			35	2 - 290	40	8 - 540							6	< 2 - 20	735	106 - 21960
[51]	Pakistan	31	Houses			0.57	< 0.2 - 3.5	0.6	< 0.2 - 2.8	0.08	< 0.2 - 0.6	0.24	< 0.2 - 8.9	0.1	< 0.2 - 0.5	0.75	< 0.2 - 64.5	19.7	< 2 - 1465
[52]	Sweden	18	Houses	0.78	< LOQ - 12	38	8.5 - 250	25	< LOQ - 130	5.5	0.85 - 33	6	0.96 - 14	2.9	0.61 - 8.3	3	1.1 - 31	520	190 - 9300
	Sweden	19	Houses	0.19	< LOQ - 2.2	15	1.5 - 47	13	0.074 - 68	2.7	0.78 - 13	2.2	< LOQ - 12	1.5	0.49 - 10	1.5	< LOQ - 8.8	280	110 - 6600
[53]	Germany	6	Houses			2.1[(c)]	1.6 - 3.6	2.6[(c)]	1.5 - 4							1.5[(c)]	0.1 - 6.5	45[(c)]	21 - 72
[54]	Sweden	10	Houses	1.3	< 0.1 - 5.6	42	< 0.5 - 230	52	< 1 - 140			6.6	0.61 - 23			12	< 0.7 - 49	320	51 - 3600
	Sweden	34	Houses	0.8	< 0.1 - 9.2	37	< 0.5 - 280	66	< 1 - 1200			7.8	< 0.2 - 410			11	< 0.7 - 110	1100	< 50 - 100000

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[55]	Romania	47	Houses															275	
[56]	US	16	Houses	26	5 - 270	2300	270 - 23000	2200	280 - 24000	520	56 - 4900	250	2 - 2400	240	22 - 1800	28	9 - 770	1400	580 - 15000
	US	16	Houses	14	3 - 310	1000	140 - 17000	1100	190 - 25000	240	37 - 11000	150	21 - 7800	110	17 - 6700	18	3 - 920	1200	110 - 8500
[57]	Czech Republic	25	Houses	< 0.1	< 0.1 - 11	8.9	< 0.1 - 398.2	11.6	< 0.1 - 95.4	< 0.1	< 0.1 - 22.6	< 0.3	< 0.3 - 5	< 0.3		3.9	< 0.8 - 457.3	375.4	40.69 - 5480.9
[58]	Germany	5	Houses	< 80[(c)]		< 50[(c)]		30[(c)]	< 1 - 84.1	< 2[(c)]	< 2 - 15.8	< 2[(c)]	< 2 - 20.5	< 2[(c)]	< 2 - 8.4	< 2[(c)]	< 2 - 394.4	343.6[(c)]	11.5 - 636.8
[59]	Spain	5	Houses	3.9[(d,e)]	< LOQ - 4.5	42[(e)]	3.7 - 358	135[(e)]	5.3 - 950	24[(e)]	< LOQ - 156	< LOQ[(e)]	< LOQ - 98	16[(e)]	< LOQ - 99				
[60]	Sweden	6	Houses	0.905[(e)]	0.14 - 4.2	19.5[(e)]	6.4 - 260	29.5[(e)]	14 - 290	4.5[(e)]	1.9 - 64	5.6[(e)]	4.6 - 26	2.6[(e)]	2.2 - 20	14.5[(e)]	< LOD - 24	985[(e)]	500 - 5200
[61]	Canada	116	Houses	4.5	0.4 - 77	280	< 0.2 - 4900	350	< 0.45 - 7600	67	< 0.02 - 1300	42	< 2.1 - 1100	25	< 0.5 - 550	14	0.8 - 270	1300	< 4.4 - 43000

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[62]	US	81	Houses	11[(b)]	< 1.5 - 277	870[(b)]	55 - 24720	919[(b)]	8 - 36210	176[(b)]	9 - 10230	88[(b)]	7 - 3407	74[(b)]	5 - 3061	8.6[(b)]	< 1.8 - 162	2574[(b)]	441 - 761300
[63]	Romania, Spain and Belgium	12	Houses	0.12[(e)]	< 0.04 - 0.87	2.66[(e)]	0.76 - 135	4.05[(e)]	0.75 - 232	0.76[(e)]	< 0.24 - 24.9	1.46[(e)]	0.12 - 23.8	0.45[(e)]	0.07 - 10.7	1.3[(e)]	0.32 - 15.1	171.5[(e)]	25.9 - 111900
[64]	Kuwait	15	Houses	0.4	< 0.2 - 28	9.5	1.3 - 4750	12	1.4 - 9390	2.3	0.3 - 2210	2.4	0.3 - 1470	1.3	0.2 - 1125	1.9	0.5 - 32	310	81 - 1250
	Pakistan	15	Houses	< 0.1	< 0.1 - 0.2	1.3	< 0.2 - 3.5	1.7	0.2 - 5.1	0.3	< 0.2 - 0.8	0.6	< 0.2 - 3	0.4	< 0.2 - 0.7	1.5	< 0.2 - 12	140	25 - 2140
[65]	New Zealand	33	Houses	0.6	0.1 - 1.3	24.2	0.3 - 98	31.5	3.3 - 219.1	6.4	0.3 - 41.1	4.6	0.3 - 58.9	3.7	0.3 - 19.8	2.7	0.3 - 238.4	598	28.8 - 27394.3
	New Zealand	16	Houses	0.8	0.1 - 7.7	46.3	6.5 - 288.4	41.8	8.1 - 540.3	9.8	0.3 - 94.1	6.7	0.3 - 58.2	3.1	0.3 - 43.1	6.3	0.3 - 21.1	1018	105.9 - 21956.2
[66]	US	38	Houses			< 1049 (Penta-BDEs)		< 1049 (Penta-BDEs)		< 1049 (Penta-BDEs)						< 30.5 (Octa-BDEs)		< 1800 (Deca-BDEs)	

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[67]	Korea	46	Houses															1200	40 - 11000
[68]	China	11	Houses, offices, electronics shops.	< 1.3[(e)] (Tri-BDE)		< 8[(e)] (Tetra-BDE)		< 9.6[(e)] (Penta-BDE)		< 9.6[(e)] (Penta-BDE)		< 3.8[(e)] (Hexa-BDE)		< 3.8[(e)] (Hexa-BDE)		< 4.7[(e)] (Hepta-BDE)		550[(e)]	99 - 1300
[69]	Australia	30	Houses	0.69	< LOR - 10	36.85	2.55 - 391	56.75	2.93 - 372	9.05	0.52 - 71.2	6.41	< LOR - 59.9	5.12	< LOR - 31.7	4.61	0.53 - 44	415	< LOR - 82200
[70]	US	292	Houses	24		1500		2400		400		310		180		28		2300	
	US	203	Houses	20		1300		2100		330		290		150		17		2500	
[11]	Japan	14	Offices	1.14	0.49 - 67	30.5	4.3 - 580	38	3.1 - 810	6.85	0.7 - 130	15.5	3.3 - 100	5.15	0.96 - 68	20	5 - 280	1100	150 - 17000
[20]	UK	18	Offices	< LOD	< LOD - 11	23	2.6 - 380	65	4.2 - 490	3.2	< LOD - 79	8.7	< LOD - 99	5.1	< LOD - 38	8.3	< LOD - 24	6200	620 - 280000

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[28]	China	12	Offices	0.87	0.53 - 1.76	8.10	5.21 - 13.18	8.03	5.13 - 10.38	1.09	0.53 - 1.85	3.05	1.97 - 7.53	1.54	0.84 - 2.24	6.77	4.26 - 8.7	3103	1705 - 4374
[35]	Australia	4	Offices			56[(e)]	47 - 210	86.5[(e)]	49 - 294	15[(e)]	9 - 61	22.5[(e)]	6 - 34	8.5[(e)]	4 - 25	36.5[(e)]	12 - 64	861[(e)]	401 - 2230
[71]	US	9	Offices	3	< LOD - 87	978	130 - 6400	1760	486 - 10600	399	73 - 1820	48	< LOD - 627	78	17 - 951	30	< LOD - 5060	1	< LOD - 29000
[39]	Belgium	10	Offices	2.1	max. > 5.3 (P95)	21.1	max. > 61.5 (P95)	45.4	max. > 133 (P95)	6.8	max. > 20.3 (P95)	12.1	max. > 663 (P95)	5.5	max. > 87.1 (P95)	23.8	max. > 3090 (P95)	443	69 - 11574
[40]	China	12	Offices	0.87	0.53 - 1.76	8.1	5.21 - 13.18	8.03	5.13 - 10.38	1.09	0.53 - 1.85	3.05	1.97 - 7.53	1.54	0.84 - 2.24	6.77	4.26 - 8.70	3103	1705 - 4374
[72]	US	31	Offices	7.5[(b,d)]	< 0.4 - 207	697[(b)]	36.8 - 19494	915[(b)]	< 0.4 - 32831	195[(b)]	12.7 - 8672	138[(b)]	11.1 - 5973	115[(b)]	7.6 - 5202	81.2[(b)]	14.9 - 12970	4204[(b)]	912 - 106204
[53]	Germany	10	Offices			19[(c)]	5.7 - 50	7.7[(c)]	3.3 - 20							1.8[(c)]	< 0.2 - 9.9	120[(c)]	28 - 310

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[54]	Sweden	10	Offices	1.2	< 0.1 - 5.4	52	14 - 390	92	14 - 770			23	4.3 - 100			55	15 - 160	780	540 - 12000
[73]	South Africa	16	Offices			44	< LOD - 81.9	76.5	< LOD - 127.7			< LOD	< LOD - 12.5					< LOD	< LOD - 571
[74]	US	31	Offices	7.5[b,d]	< 0.4 - 207	697[b]	36.8 - 19494	915[b]	< 0.4 - 32831	195[b]	12.7 - 8672	138[b]	11.1 - 5973	115[b]	7.6 - 5202	81.2[b]	14.9 - 12970	4204[b]	912 - 106204
[20]	UK	20	Cars	< LOD	< LOD - 43	54	19 - 7500	100	23 - 8000	17	< LOD - 2300	11	< LOD - 1500	11	< LOD - 1900	7.8	< LOD - 67	100000	12000 - 2600000
[26]	US	12	Cars	13	max. 310	1800	max. 30000	2600	max. 63000	790	max. 8100	77	max. 7200	120	max. 2000	73	max. 31000	3100	max. 210000000
[75]	US	60	Cars	118	< 3.2 - 763	880	139 - 13600	1130	201 - 22900	211	32 - 3870	163	43 - 16600	105	21 - 2050	73	< 3.8 - 5650	48100	4380 - 3570000
[37]	US	2	Cars	3.45[f]	2.4 - 4.5	370.15[f]	343.7 - 396.6	490.5[f]	448.6 - 532.4	82.05[f]	76.4 - 87.7	33.85[f]	27.7 - 40	28.6[f]	23 - 34.2	3.75[f]	3.5 - 4	2721.5[f]	2621 - 2822

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[38]	Portugal	9	Cars	4[(e)]	< LOQ - 46	88[(e)]	26 - 1549	126[(e)]	13 - 3104	23[(e)]	2 - 914	23[(e)]	1 - 937	12[(e)]	2 - 697	6[(e)]	3 - 28	1119[(e)]	98 - 17122
[76]	UK	14	Cars			100	28 - 3624	130	45 - 4260	17	< LOQ- 695	14	< LOQ - 400	10	< LOQ - 367	6	< LOQ - 59	190000	28126 - 784129
	UK	14	Cars			26	5 - 71	50	14 - 101	6.5	< LOQ - 46	6.5	< LOQ - 41	1.5	< LOQ - 36	1.5	< LOQ - 11	2700	183 - 11065
[53]	Germany	12	Cars			17[(c)]	2.1 - 47	32[(c)]	1.3 - 88							3.7[(c)]	< 0.2 - 17	940[(c)]	220 - 3100
[54]	Sweden	4	Cars	0.2	< 0.1 - 0.4	7.4	0.56 - 22	11	1.5 - 30			3.1	0.25 - 6.7			2.2	< 0.7 - 6.7	1300	50 - 28000
[57]	Czech Republic	27	Cars	< 0.1		2.2	< 0.1 - 284.3	< 0.1	< 0.1 - 475.6	< 0.1	< 0.1 - 19.5	< 0.3	< 0.3 - 42.9	< 0.3	< 0.3 - 29.8	< 0.8	< 0.8 - 15.4	168.5	< 5 - 325775.2
[64]	Kuwait	15	Cars	0.2	< 0.2 - 2	5.8	0.6 - 14.5	8.5	1 - 62	1.5	< 0.2 - 17	1.5	< 0.2 - 70	1.1	< 0.2 - 6	1	< 0.2 - 3.6	665	107 - 136900
	Pakistan	15	Cars	< 0.1	< 0.1 - 1.3	1.2	< 0.2 - 7.5	1.7	0.4 - 8	0.3	< 0.2 - 1.5	0.9	< 0.2 - 2.2	0.3	< 0.2 - 0.8	1.2	< 0.2 - 8.5	625	25 - 260700

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[77]	UK	43	Daycare centers and primary schools	< 1	< 1-25	26	1.6 - 120	36	1.1 - 270	6.6	< 1-50	10	< 2 - 310	2.8	< 2 - 26	1.2	< 2 - 48	5000	49 - 88000
[78]	South Korea	13	Elementary school	0.297		4.09		6.44		1.16		3.81		1.88		13.3		1360	
	South Korea	6	Elementary school	0.271		12.2		50.9		3.54		11.3		2.76		6.85		833	
[79]	South Korea	24	Playrooms	18.6[(c)]	< LOD - 55.81	45.21 [(c)]	5.79 - 118.03	23.73 [(c)]	8.59 - 50.91	3.48[(c)]	1.38 - 6.35	26.87 [(c)]	17.3 - 44.3	5.58[(c)]	2.93 - 10.07	125.96[(c)]	105.21 - 165.94	1307.15[(c)]	705.36 - 2328.69
	South Korea	24	Daycare center	0.82[(c)]	< LOD - 2.93	77.03 [(c)]	5.82 - 260.1	100.64[(c)]	7.35 - 342.63	17.81 [(c)]	1.19 - 61.62	31.52 [(c)]	3.01 - 98.49	18.37 [(c)]	1.02 - 63.97	345.63[(c)]	13.71 - 1177.25	7742.29[(c)]	525.44 - 26607.3
	South Korea	24	Kindergarten	< LOD[(c)]		7.13[(c)]	2.85 - 13.82	8.79[(c)]	4.93 - 13.59	1.79[(c)]	0.78-3.48	43.25 [(c)]	7.25 - 68.58	6.54[(c)]	1.31 - 10.4	259.45[(c)]	37.14 - 409.37	5831.09[(c)]	1899.1 - 12102.8

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
	South Korea	24	Indoor playground	26.15 (c)	< LOD - 58.3	103.57 (c)	3.25 - 209.88	48.52 (c)	6.62 - 96.43	5.09 (c)	1 - 15.17	43.05 (c)	8.39 - 83.72	14.32 (c)	1.75 - 34.9	331.58 (c)	40.75 - 628.4	16329.6 (c)	912.27 - 31757
[54]	Sweden	10	Day care centers	2.8	< 0.1 - 8.2	120	31 - 910	110	42 - 550			12	6.1 - 19			6.5	2.7 - 15	580	180 - 3500
[80]	Philippines	8	University of the Philippines															2172	1103 – 4117
[81]	China	9	Shanghai University	0.19	< LOD - 0.51	2.53	0.49 - 9.09	2.52	0.68 - 10.5	0.48	< LOD - 1.36	1.03	0.15 - 2.91	0.91	0.55 - 3.54	3.5	0.02 - 7.36	585	152 - 1350
[82]	China	1	Shanghai University	25.4 (a)		14.5 (a)		25.1 (a)		3.6 (a)		4.9 (a)		3.6 (a)		10.4 (a)		1828.5 (a)	
	China	1	Shanghai University	14.1 (a)		337.5 (a)		503 (a)		114.6 (a)		19.7 (a)		30.9 (a)		12.7 (a)		1091.2 (a)	
[83]	Japan	8	Commercial hotel	0.53 (e)	0.21 - 3.4	4.75 (e)	0.9 - 22	5.95 (e)	0.6 - 15	0.80 (e)	< 0.1 - 2.9	1.75 (e)	0.4 - 5.3	0.56 (e)	0.17 - 1.3	3 (e)	0.6 - 24	1080 (e)	< 10 - 1500

REF	Location	n	Origin	BDE28 (Median; Range)		BDE47 (Median; Range)		BDE99 (Median; Range)		BDE100 (Median; Range)		BDE153 (Median; Range)		BDE154 (Median; Range)		BDE183 (Median; Range)		BDE209 (Median; Range)	
[46]	China	55	Workplaces	23.3	1.69 - 101	109	1.98 - 1586	186	6.35 - 10098	144	10.5 - 458	24.5	< LOD - 908	18.2	< LOD - 876	27.8	2.63 - 133	1401	103 - 37440
[51]	Pakistan	12	Mosques			2.45	0.2 - 7.95	2.99	0.2 - 38	0.56	< 0.2 - 3.05	0.48	< 0.2 - 4.8	0.33	< 0.2 - 2.6	0.73	< 0.2 - 6.95	39.8	4.7 - 297
[26]	US	12	Garages	3	max. 74	160	max. 7900	280	max. 11000	39	max. 1800	6	max. 150	7	max. 950	8	max. 3300	7	max. 3900000

(a) The reported concentrations refer to a single sample or a pooled sample.
(b) Median values were not reported, reported geometric means were considered in this table.
(c) Median values were not reported, reported means were considered in this table.
(d) BDE-28 and -33 are listed together, however the reported concentrations were considered as BDE-28 concentrations, once the BDE-33 is a minor congener both in technical mix and in environmental and biological matrices.
(e) Median values were not reported, we calculated medians from reported data considering half of the limit of detection (LOD) or limit of quantification (LOQ***);*** LOQ was considered zero in the case of Cunha et al, 2010; Martínez-Moral and Tena, 2012 “not detected” was considered zero.
(f) Median values were not reported, we calculated means from reported data once the number of samples was limited

Table 2. Median and range concentrations (ng g^{-1}) of total hexabromocyclododecane and respective diastereomers in indoor dust reported worldwide in the last 10 years.

REF	Location	n	Origin	∑HBCD (median; range)		α-HBCD (median; range)		β-HBCD (median; range)		γ-HBCD (median; range)	
[3]	UK	10	Houses	3250	940 - 6900						
	Denmark	1	Houses	1000[(a)]							
	Finland	1	Houses	790[(a)]							
[4]	Germany	1	Houses	1200[(a)]							
	Spain	4	Houses	225	190 - 850						
	France	8	Houses	485	77 - 1600						
	Italy	1	Houses	250[(a)]							
[5]	Belgium	23	Houses and offices	< 20	< 20 - 57554						
[84]	UK	45	Houses	1300	140 - 140000	380	22 - 66000	93	9 - 26000	670	70 - 75000
[85]	UK	31	Houses	730	140 - 110000	170	22 - 66000	66	9 - 7800	440	70 - 37000
	US	13	Houses	390	110 - 4000	80	17 - 1800	28	6 - 300	300	79 - 2000
	Canada	8	Houses	640	64 - 1300	300	25 - 670	72	6 - 130	230	34 - 470
[86]	US	20	Houses	230[(b)]	< 4.5 - 130200						

REF	Location	n	Origin	∑HBCD (median; range)		α-HBCD (median; range)		β-HBCD (median; range)		γ-HBCD (median; range)	
	US	20	Houses	144[(b)]	< 4.5 - 9710						
	US	20	Houses	282[(b)]	21 - 35100						
[87]	UK	21	Houses	2401[(d)]	228 - 140774	876[(d)]	112 - 40653	300[(d)]	25 - 25513	886[(d)]	80 - 74607
[88]	UK	3	Houses	4179[(e)]	238 - 10940	1881[(e)]	58.4 - 5040	570[(e)]	23.6 - 1530	1702[(e)]	154 - 4280
[89]	Belgium	16	Houses	114	33 - 758	69	22 - 481	14	4 - 87	31	7 - 190
[34]	Japan	2	Houses	6570[(c)]	140 - 13000						
[39]	Belgium	45	Houses	130	5 - 42692						
[42]	Philippines	25	Houses	8.1	0.71 - 99	4.6	0.62 - 30	1	< LOD - 10	4	< LOD - 66
[90]	Belgium	43	Houses	140.33	max. > 4092.74 (P95)						
[45]	Germany/US	26	Houses	166	30 - 15000						

REF	Location	n	Origin	∑HBCD (median; range)		α-HBCD (median; range)		β-HBCD (median; range)		γ-HBCD (median; range)	
[50]	New Zealand	50	Houses	190	20 - 4100	99	3 - 1790	12	3 - 270	96	8 - 3020
[52]	Sweden	18	Houses	8.9	< LOQ - 62						
	Sweden	19	Houses	86	< LOQ - 95000						
[54]	Sweden	10	Houses	100	15 - 990						
	Sweden	34	Houses	45	< 3 - 2400						
[55]	Romania	47	Houses	250							
[56]	US	16	Houses	190	82 - 6800	62	31 - 710	18	8 - 330	94	29 - 6700
	US	16	Houses	160	39 - 1800	62	17 - 910	16	7 - 230	73	13 - 790
[57]	Czech Republic	25	Houses	92.6	< 0.3 - 949.5	25.9	< 0.3 - 275	7.1	< 0.3 - 57.3	61.2	< 0.3 - 739.5
[58]	Germany	5	Houses			162.4 (c)	31.5 - 275.8	35.23 (c)	7.9 - 64	97.4 (c)	13.5 - 164.1
[60]	Sweden	6	Houses	151.5	100 - 4100	93	59 - 2900	24.5	20 - 540	38.5	14 - 660
[61]	Canada	116	Houses	270	20 - 4700						
[63]	Romania, Spain and Belgium	12	Houses	207(d)	41.5 - 2185	109.4(d)	12.2 - 1550	10.3(d)	< 3 - 442	25.95(d)	9.8 - 1101

REF	Location	n	Origin	∑HBCD (median; range)		α-HBCD (median; range)		β-HBCD (median; range)		γ-HBCD (median; range)	
[66]	US	38	Houses	246	max. 1999						
[84]	UK	28	Offices	760	90 - 6600	220	15 - 2900	84	11 - 1300	470	36 - 3700
[85]	UK	6	Offices	650	90 - 3600	100	15 - 630	75	11 - 380	470	65 - 2600
[87]	UK	21	Offices	1602(d)	279 - 4004	587(d)	97 - 2866	176(d)	21 - 545	621(d)	69 - 2579
[88]	UK	3	Offices	2887(e)	2080 - 4220	1219(e)	838 - 1600	328(e)	224 - 464	1330(e)	448 - 2520
[39]	Belgium	10	Offices	367	256 - 1153						
[90]	Belgium	10	Offices	1288.73	max. > 5836.79 (P95)						
[54]	Sweden	10	Offices	300	190 - 5700						
[84]	UK	20	Cars	13000	190 - 69000	2000	54 - 8800	740	16 - 5200	9600	27 - 56000
[87]	UK	12	Cars	14262.5(d)	194 - 55822	1230.5(d)	54 - 8838	358.5(d)	16 - 4251	9650.5(d)	124 - 47737
[76]	UK	14	Cars	9200	1241 - 23722	3000	311 - 8611	1100	76 - 3037	4800	792 - 12074
	UK	14	Cars	1300	198 - 3097	280	34 - 801	130	19 - 283	860	145 - 2154

REF	Location	n	Origin	∑HBCD (median; range)		α-HBCD (median; range)		β-HBCD (median; range)		γ-HBCD (median; range)	
[54]	Sweden	4	Cars	54	6.8 - 170						
[57]	Czech Republic	27	Cars	32.7	< 0.3 - 241.4	9.2	< 0.3 - 45.4	< 0.3	< 0.3 - 44.3	25	< 0.3 - 151.6
[84]	UK	4	Pubs and restaurant	2700	2300 - 3200	1000	810 - 1200	310	270 - 420	1300	1100 - 1700
[83]	Japan	8	Commercial hotel	735	72 - 1300						
[77]	UK	43	Daycare centers and primary schools	4100	72 - 89000	1400	24 - 10000	550	14 - 6700	1700	34 - 72000
[54]	Sweden	10	Daycare centers	340	190 - 1600						

(a) The reported concentrations refer to a single sample or a pooled sample.
(b) Median values were not reported, reported geometric means were considered in this table.
(c) Median values were not reported, reported means were considered in this table.
(d) Median values were not reported, we calculated medians from reported data
(e) Median values were not reported, we calculated means from reported data once the number of samples was limited.

References

[1] Knoth, W.; Mann, W.; Meyer, R.; Nebhuth, J., Brominated diphenyl ether in indoor dust. In *Organohalogen Compounds*: Boston, MA, **2003**; Vol. 61, pp. 207-210.

[2] Rudel, R. A.; Camann, D. E.; Spengler, J. D.; Korn, L. R.; Brody, J. G., Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environ. Sci. Technol.*, **2003**, *37*(20), 4543-4553.

[3] Santillo, D.; Labunska, I.; Davidson, H.; Johnston, P.; Strutt, M.; Knowles, O. *Consuming Chemicals: Hazardous Chemicals in House Dust as an Indicator of Chemical Exposure in the Home*; Greenpeace Research Laboratories, Department of Biological Sciences, University of Exeter, Exeter UK, **2003**.

[4] Santillo, D.; Labunska, I.; Fairley, M.; Johnston, P. *Consuming Chemicals #2: Hazardous Chemicals in House Dust as Indicators of Chemical Exposure in the Home*; Greenpeace Research Laboratories, Department of Biological Sciences, University of Exeter, Exeter, UK, **2003**.

[5] Al Bitar, F. *Hazardous Chemicals in Belgian House Dust;* Greenpeace, **2004**.

[6] Schecter, A.; Päpke, O.; Joseph, J. E.; Tung, K. C., Polybrominated diphenyl ethers (PBDEs) in U.S. computers and domestic carpet vacuuming: Possible sources of human exposure. *J. Toxicol. Environ. Health A*, **2005**, *68*(7), 501-513.

[7] Stapleton, H. M.; Dodder, N. G.; Offenberg, J. H.; Schantz, M. M.; Wise, S. A., Polybrominated diphenyl ethers in house dust and clothes dryer lint. *Environ. Sci. Technol.*, **2005**, *39*(4), 925-931.

[8] Wilford, B. H.; Shoeib, M.; Harner, T.; Zhu, J.; Jones, K. C., Polybrominated Diphenyl Ethers in Indoor Dust in Ottawa, Canada: Implications for Sources and Exposure. *Environ. Sci. Technol.*, **2005,** *39*(18), 7027-7035.

[9] Gevao, B.; Al-Bahloul, M.; Al-Ghadban, A. N.; Al-Omair, A.; Ali, L.; Zafar, J.; Helaleh, M., House dust as a source of human exposure to polybrominated diphenyl ethers in Kuwait. *Chemosphere*, **2006,** *64*(4), 603-608.

[10] Regueiro, J.; Llompart, M.; García-Jares, C.; Cela, R., Determination of polybrominated diphenyl ethers in domestic dust by microwave-assisted solvent extraction and gas chromatography-tandem mass spectrometry. *J. Chromatogr. A*, **2006,** *1137*(1), 1-7.

[11] Suzuki, G.; Nose, K.; Takigami, H.; Takahashi, S.; Sakai, S.-I., PBDEs and PBDD/Fs in house and office dust from Japan. In *Organohalogen Compounds*: Oslo, Norway, **2006**; Vol. 68, pp 1843–1846.

[12] Pless-Mulloli, T.; Schecter, A.; Schilling, B.; Paepke, O., Levels of PBDE in household dust and lint in the UK, Germany and the USA. In *Organohalogen Compounds*: Oslo, Norway, **2006**; Vol. 68, pp 495-498.

[13] Karlsson, M.; Julander, A.; van Bavel, B.; Hardell, L., Levels of brominated flame retardants in blood in relation to levels in household air and dust. *Environ. Int.*, **2007**, *33*(1), 62-69.

[14] Regueiro, J.; Llompart, M.; Garcia-Jares, C.; Cela, R., Factorial-design optimization of gas chromatographic analysis of tetrabrominated to decabrominated diphenyl ethers. Application to domestic dust. *Anal. Bioanal. Chem.*, **2007**, *388*(5-6), 1095-1107.

[15] Tan, J.; Cheng, S. M.; Loganath, A.; Chong, Y. S.; Obbard, J. P., Polybrominated diphenyl ethers in house dust in Singapore. *Chemosphere*, **2007,** *66*(6), 985-992.

[16] Wu, N.; Herrmann, T.; Paepke, O.; Tickner, J.; Hale, R.; Harvey, E.; La Guardia, M.; McClean, M. D.; Webster, T. F., Human Exposure to PBDEs: Associations of PBDE Body Burdens with Food Consumption and House Dust Concentrations. *Environ. Sci. Technol.*, **2007**, *41*(5), 1584-1589.

[17] Kono, Y.; Fukuzawa, E.; Miyazaki, M.; Nomura, T.; Komatsu, K., Polybrominated dioxins (PBDDs/DFs) and polybrominated diphenyl ethers (PBDEs) in house dust in ordinary homes. In *Organohalogen Compounds*: Tokyo, Japan, **2007**; Vol. 69, pp 2781-2784.

[18] Allen, J. G.; McClean, M. D.; Stapleton, H. M.; Webster, T. F., Critical factors in assessing exposure to PBDEs via house dust. *Environ. Int.*, **2008**, *34*(8), 1085-1091.

[19] Dirtu, A. C.; Ravindra, K.; Roosens, L.; van Grieken, R.; Neels, H.; Blust, R.; Covaci, A., Fast analysis of decabrominated diphenyl ether using low-pressure gas chromatography–electron-capture negative ionization mass spectrometry. *J. Chromatogr. A*, **2008**, *1186*(1-2), 295-301.
[20] Harrad, s.; Ibarra, C.; Abdallah, M. A. E.; Boon, R.; Neels, H.; Covaci, A., Concentrations of brominated flame retardants in dust from United Kingdom cars, homes, and offices: Causes of variability and implications for human exposure. *Environ. Int.*, **2008**, *34*(8), 1170-1175.
[21] Harrad, S.; Ibarra, C.; Diamond, M.; Melymuk, L.; Robson, M.; Douwes, J.; Roosens, L.; Dirtu, A. C.; Covaci, A., Polybrominated diphenyl ethers in domestic indoor dust from Canada, New Zealand, United Kingdom and United States. *Environ. Int.*, **2008**, *34*(2), 232-238.
[22] Hwang, H.-M.; Park, E.-K.; Young, T. M.; Hammock, B. D., Occurrence of endocrine-disrupting chemicals in indoor dust. *Sci. Total Environ.*, **2008**, *404*(1), 26-35.
[23] Sjödin, A.; Päpke, O.; McGahee, E.; Focant, J.-F.; Jones, R. S.; Pless-Mulloli, T.; Toms, L.-M. L.; Herrmann, T.; Müller, J.; Needham, L. L.; Patterson Jr, D. G., Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries. *Chemosphere*, **2008**, *73*(1, Supplement 1), S131-S136.
[24] Wang, D.; Atkinson, S.; Hoover-Miller, A.; Shelver, W. L.; Li, Q. X., Simultaneous use of gas chromatography/ion trap mass spectrometry - Electron capture detection to improve the analysis of bromodiphenyl ethers in biological and environmental samples. *Rapid. Commun. Mass. Spectrom.*, **2008**, *22*(5), 647-656.
[25] Zota, A. R.; Rudel, R. A.; Morello-Frosch, R. A.; Brody, J. G., Elevated house dust and serum concentrations of PBDEs in California: Unintended consequences of furniture flammability standards? *Environ. Sci. Technol.*, **2008**, *42*(21), 8158-8164.
[26] Batterman, S. A.; Chernyak, S.; Jia, C.; Godwin, C.; Charles, S., Concentrations and emissions of polybrominated diphenyl ethers from U.S. houses and garages. *Environ. Sci. Technol.*, **2009**, *43*(8), 2693-2700.
[27] Fromme, H.; Körner, W.; Shahin, N.; Wanner, A.; Albrecht, M.; Boehmer, S.; Parlar, H.; Mayer, R.; Liebl, B.; Bolte, G., Human exposure to polybrominated diphenyl ethers (PBDE), as evidenced by data from a duplicate diet study, indoor air, house dust, and biomonitoring in Germany. *Environ. Int.*, **2009**, *35*(8), 1125-1135.
[28] Huang, Y. M.; Chen, L. G.; Wen, L. J.; Xu, Z. C.; Peng, X. C.; Ye, Z. X.; Zeng, M., Distribution and possible sources of PBDEs in indoor dust in Guangzhou City. *China Environ. Sci.*, **2009**, *29*(11), 1147-1152.
[29] Imm, P.; Knobeloch, L.; Buelow, C.; Anderson, H. A., Household Exposures to Polybrominated Diphenyl Ethers (PBDEs) in a Wisconsin Cohort. *Environ. Health Perspect.*, **2009**, *117*(12), 1890–1895.
[30] Johnson-Restrepo, B.; Kannan, K., An assessment of sources and pathways of human exposure to polybrominated diphenyl ethers in the United States. *Chemosphere*, **2009**, *76*(4), 542-548.
[31] Meeker, J. D.; Johnson, P. I.; Camann, D.; Hauser, R., Polybrominated diphenyl ether (PBDE) concentrations in house dust are related to hormone levels in men. *Sci. Total Environ.*, **2009**, *407*(10), 3425-3429.
[32] Muenhor, D.; Harrad, S.; Evans, T. J., Polybrominated diphenyl ethers (PBDEs) in air and dust from electronic waste storage facilities and houses in Thailand: implications for human exposure. In *Organohalogen Compounds:* Beijing, China, **2009**; Vol. 71, pp 16-20.
[33] Roosens, L.; Abdallah, M. A. E.; Harrad, S.; Neels, H.; Covaci, A., Factors influencing concentrations of polybrominated diphenyl ethers (PBDEs) in students from Antwerp, Belgium. *Environ. Sci. Technol.*, **2009**, *43*(10), 3535-3541.
[34] Takigami, H.; Suzuki, G.; Hirai, Y.; Sakai, S. i., Brominated flame retardants and other polyhalogenated compounds in indoor air and dust from two houses in Japan. *Chemosphere*, **2009**, *76*(2), 270-277.
[35] Toms, L. M. L.; Bartkow, M. E.; Symons, R.; Paepke, O.; Mueller, J. F., Assessment of polybrominated diphenyl ethers (PBDEs) in samples collected from indoor environments in South East Queensland, Australia. *Chemosphere*, **2009**, *76*(2), 173-178.

[36] Toms, L.-M. L.; Hearn, L.; Kennedy, K.; Harden, F.; Bartkow, M.; Temme, C.; Mueller, J. F., Concentrations of polybrominated diphenyl ethers (PBDEs) in matched samples of human milk, dust and indoor air. *Environ. Int.*, **2009**, *35*(6), 864-869.
[37] Wei, H.; Turyk, M.; Cali, S.; Dorevitch, S.; Erdal, S.; Li, A., Particle size fractionation and human exposure of polybrominated diphenyl ethers in indoor dust from Chicago. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.*, **2009**, *44*(13), 1353-1361.
[38] Cunha, S. C.; Kalachova, K.; Pulkrabova, J.; Fernandes, J. O.; Oliveira, M. B. P. P.; Alves, A.; Hajslova, J., Polybrominated diphenyl ethers (PBDEs) contents in house and car dust of Portugal by pressurized liquid extraction (PLE) and gas chromatography-mass spectrometry (GC-MS). *Chemosphere*, **2010**, *78*(10), 1263-1271.
[39] D'Hollander, W.; Roosens, L.; Covaci, A.; Cornelis, C.; Reynders, H.; Campenhout, K. V.; Voogt, P. d.; Bervoets, L., Brominated flame retardants and perfluorinated compounds in indoor dust from homes and offices in Flanders, Belgium. *Chemosphere*, **2010**, *81*(4), 478-487.
[40] Huang, Y.; Chen, L.; Peng, X.; Xu, Z.; Ye, Z., PBDEs in indoor dust in South-Central China: Characteristics and implications. *Chemosphere*, **2010**, *78*(2), 169-174.
[41] Johnson, P. I.; Stapleton, H. M.; Sjodin, A.; Meeker, J. D., Relationships between Polybrominated Diphenyl Ether Concentrations in House Dust and Serum. *Environ. Sci. Technol.*, **2010**, *44*(14), 5627-5632.
[42] Malarvannan, G.; Isobe, T.; Sudaryanto, A.; Takahashi, S.; Prudente, M.; Tanabe, S., Brominated flame retardants in human breast milk and house dust from the Philippines: implications for human exposure. In *Organohalogen Compounds*: San Antonio, Texas, **2010**; Vol. 72, pp 1742-1745.
[43] Wang, J.; Ma, Y.-J.; Chen, S.-J.; Tian, M.; Luo, X.-J.; Mai, B.-X., Brominated flame retardants in house dust from e-waste recycling and urban areas in South China: Implications on human exposure. *Environ. Int.*, **2010**, *36*(6), 535-541.
[44] Whitehead, T.; Holden, A.; Odion, Z.; Vista, P.; Brown, F.; Metayer, C.; Rappaport, S.; Buffler, P.; Petreas, M., A method for the determination of PBDEs, PCBs, Pesticides and PAHs in dust. In *Organohalogen Compounds*: San Antonio, Texas, **2010**; Vol. 72, pp 181-184.
[45] Abb, M.; Stahl, B.; Lorenz, W., Analysis of brominated flame retardants in house dust. *Chemosphere*, **2011**, *85*(11), 1657-1663.
[46] Kang, Y.; Wang, H. S.; Cheung, K. C.; Wong, M. H., Polybrominated diphenyl ethers (PBDEs) in indoor dust and human hair. *Atmos. Environ.*, **2011**, *45*(14), 2386-2393.
[47] Quirós-Alcalá, L.; Bradman, A.; Nishioka, M.; Harnly, M. E.; Hubbard, A.; McKone, T. E.; Eskenazi, B., Concentrations and loadings of polybrominated diphenyl ethers in dust from low-income households in California. *Environ. Int.*, **2011**, *37*(3), 592-596.
[48] Vorkamp, K.; Thomsen, M.; Frederiksen, M.; Pedersen, M.; Knudsen, L. E., Polybrominated diphenyl ethers (PBDEs) in the indoor environment and associations with prenatal exposure. *Environ. Int.*, **2011**, *37*(1), 1-10.
[49] Zheng, J.; Luo, X.-J.; Yuan, J.-G.; Wang, J.; Wang, Y.-T.; Chen, S.-J.; Mai, B.-X.; Yang, Z.-Y., Levels and sources of brominated flame retardants in human hair from urban, e-waste, and rural areas in South China. *Environ. Pollut.*, **2011**, *159*(12), 3706-3713.
[50] Ali, N.; Dirtu, A. C.; Eede, N. V. D.; Goosey, E.; Harrad, S.; Neels, H.; t Mannetje, A.; Coakley, J.; Douwes, J.; Covaci, A., Occurrence of alternative flame retardants in indoor dust from New Zealand: Indoor sources and human exposure assessment. *Chemosphere*, **2012**, *88*(11), 1276-1282.
[51] Ali, N.; Van den Eede, N.; Dirtu, A. C.; Neels, H.; Covaci, A., Assessment of human exposure to indoor organic contaminants via dust ingestion in Pakistan. *Indoor Air*, **2012**, *22*(3), 200-211.
[52] Björklund, J. A.; Sellström, U.; de Wit, C. A.; Aune, M.; Lignell, S.; Darnerud, P. O., Comparisons of polybrominated diphenyl ether and hexabromocyclododecane concentrations in dust collected with two sampling methods and matched breast milk samples. *Indoor Air*, **2012**, *22*(4), 279-288.

[53] Brommer, S.; Harrad, S.; Van Den Eede, N.; Covaci, A., Concentrations of organophosphate esters and brominated flame retardants in German indoor dust samples. *J. Environ. Monit.*, **2012**, *14*(9), 2482-2487.
[54] Thuresson, K.; Björklund, J. A.; de Wit, C. A., Tri-decabrominated diphenyl ethers and hexabromocyclododecane in indoor air and dust from Stockholm microenvironments 1: Levels and profiles. *Sci. Total Environ.*, **2012**, *414*, 713-721
[55] Dirtu, A. C.; Ali, N.; Van den Eede, N.; Neels, H.; Covaci, A., Country specific comparison for profile of chlorinated, brominated and phosphate organic contaminants in indoor dust. Case study for Eastern Romania, 2010. *Environ. Int.*, **2012**, *49*, 1-8.
[56] Dodson, R. E.; Perovich, L. J.; Covaci, A.; Van Den Eede, N.; Ionas, A. C.; Dirtu, A. C.; Brody, J. G.; Rudel, R. A., After the PBDE phase-out: A broad suite of flame retardants in repeat house dust samples from California. *Environ. Sci. Technol.*, **2012**, *46*(24), 13056-13066.
[57] Kalachova, K.; Hradkova, P.; Lankova, D.; Hajslova, J.; Pulkrabova, J., Occurrence of brominated flame retardants in household and car dust from the Czech Republic. *Sci. Total Environ.*, **2012**, *441*, 182-193.
[58] Kopp, E. K.; Fromme, H.; Völkel, W., Analysis of common and emerging brominated flame retardants in house dust using ultrasonic assisted solvent extraction and on-line sample preparation via column switching with liquid chromatography-mass spectrometry. *J. Chromatogr. A*, **2012**, *1241*, 28-36.
[59] Martínez-Moral, M. P.; Tena, M. T., Focused ultrasound solid-liquid extraction and gas chromatography tandem mass spectrometry determination of brominated flame retardants in indoor dust. *Anal. Bioanal. Chem.*, **2012**, *404*(2), 289-295.
[60] Sahlström, L.; Sellström, U.; De Wit, C. A., Clean-up method for determination of established and emerging brominated flame retardants in dust. *Anal. Bioanal. Chem.* **2012**, *404*(2), 459-466.
[61] Shoeib, M.; Harner, T.; Webster, G. M.; Sverko, E.; Cheng, Y., Legacy and current-use flame retardants in house dust from Vancouver, Canada. *Environ. Pollut.*, **2012**, *169*, 175-182.
[62] Stapleton, H. M.; Eagle, S.; Sjödin, A.; Webster, T. F., Serum PBDEs in a North Carolina toddler cohort: Associations with handwipes, house dust, and socioeconomic variables. *Environ. Health Perspect.*, **2012,** *120*(7), 1049-1054.
[63] Van den Eede, N.; Dirtu, A. C.; Ali, N.; Neels, H.; Covaci, A., Multi-residue method for the determination of brominated and organophosphate flame retardants in indoor dust. *Talanta*, **2012**, *89*, 292-300.
[64] Ali, N.; Ali, L.; Mehdi, T.; Dirtu, A. C.; Al-Shammari, F.; Neels, H.; Covaci, A., Levels and profiles of organochlorines and flame retardants in car and house dust from Kuwait and Pakistan: Implication for human exposure via dust ingestion. *Environ. Int.*, **2013**, *55*, 62-70.
[65] Coakley, J. D.; Harrad, S. J.; Goosey, E.; Ali, N.; Dirtu, A. C.; Van den Eede, N.; Covaci, A.; Douwes, J.; Mannetje, A., Concentrations of polybrominated diphenyl ethers in matched samples of indoor dust and breast milk in New Zealand. *Environ. Int.*, **2013**, *59*, 255-261.
[66] Johnson, P. I.; Stapleton, H. M.; Mukherjee, B.; Hauser, R.; Meeker, J. D., Associations between brominated flame retardants in house dust and hormone levels in men. *Sci. Total Environ.*, **2013**, *445-446*, 177-184.
[67] Lee, S.; Kannan, K.; Moon, H.-B., Assessment of exposure to polybrominated diphenyl ethers (PBDEs) via seafood consumption and dust ingestion in Korea. *Sci. Total Environ.*, **2013**, *443*, 24-30.
[68] Li, K.; Fu, S., Polybrominated Diphenyl Ethers (PBDEs) in House Dust in Beijing, China. *Bull. Environ. Contam. Toxicol.* **2013**, *4*, 382-384.
[69] Stasinska, A.; Reid, A.; Hinwood, A.; Stevenson, G.; Callan, A.; Odland, J. T.; Heyworth, J., Concentrations of polybrominated diphenyl ethers (PBDEs) in residential dust samples from Western Australia. *Chemosphere*, **2013**, *91*(2), 187-193.
[70] Whitehead, T. P.; Brown, F. R.; Metayer, C.; Park, J.-S.; Does, M.; Petreas, M. X.; Buffler, P. A.; Rappaport, S. M., Polybrominated diphenyl ethers in residential dust: Sources of variability. *Environ. Int.*, **2013**, *57–58*, 11-24.

[71] Batterman, S.; Godwin, C.; Chernyak, S.; Jia, C.; Charles, S., Brominated flame retardants in offices in Michigan, U.S.A. *Environ. Int.*, **2010**, *36*(6), 548-556.
[72] Watkins, D. J.; McClean, M. D.; Fraser, A. J.; Weinberg, J.; Stapleton, H. M.; Sjödin, A.; Webster, T. F., Exposure to PBDEs in the Office Environment: Evaluating the Relationships Between Dust, Handwipes, and Serum. *Environ. Health Perspect.*, **2011**, *119*(9), 1247-1252.
[73] Kefeni, K. K.; Okonkwo, J. O., Analysis of major congeners of polybromobiphenyls and polybromodiphenyl ethers in office dust using high resolution gas chromatography-mass spectrometry. *Chemosphere*, **2012**, *87*(9), 1070-1075.
[74] Watkins, D. J.; McClean, M. D.; Fraser, A. J.; Weinberg, J.; Stapleton, H. M.; Webster, T. F., Associations between PBDEs in office air, dust, and surface wipes. *Environ. Int.*, **2013**, *59*, 124-132.
[75] Lagalante, A. F.; Oswald, T. D.; Calvosa, F. C., Polybrominated diphenyl ether (PBDE) levels in dust from previously owned automobiles at United States dealerships. *Environ. Int.*, **2009**, *35*(5), 539-544.
[76] Harrad, S.; Abdallah, M. A.-E., Brominated flame retardants in dust from UK cars - Within-vehicle spatial variability, evidence for degradation and exposure implications. *Chemosphere*, **2011**, *82*(9), 1240-1245.
[77] Harrad, S.; Goosey, E.; Desborough, J.; Abdallah, M. A.-E.; Roosens, L.; Covaci, A., Dust from U.K. Primary School Classrooms and Daycare Centers: The Significance of Dust As a Pathway of Exposure of Young U.K. Children to Brominated Flame Retardants and Polychlorinated Biphenyls. *Environ. Sci. Technol.*, **2010**, *44*(11), 4198-4202.
[78] Wu, Q.; Baek, S. Y.; Fang, M.; Chang, Y. S., Distribution and fate of polybrominated diphenyl ethers in indoor environments of elementary schools. *Indoor Air*, **2010**, *20*(3), 263-270.
[79] Kim, H. H.; Yang, J. Y.; Jang, Y. S.; Lee, Y. J.; Lee, C. S.; Shin, D. C.; Lim, Y. W., Exposure assessment and health risk of Polybrominated Diphenyl Ether (PBDE) flame retardants in indoor environments of children's facilities in Korea. *Asian J. Atmos. Environ.*, **2011**, *5*(4), 247-262.
[80] Fulong, C. R. P.; Espino, M. P. B., Decabromodiphenyl ether in indoor dust from different microenvironments in a university in the Philippines. *Chemosphere*, **2013**, *90*(1), 42-48.
[81] Tang, L.; Lei, B.; Xu, G.; Ma, J.; Lei, J. Q.; Jin, S. Q.; Hu, G. Y.; Wu, M. H., Polybrominated Diphenyl Ethers in Human Hair from the College Environment: Comparison with Indoor Dust. *Bull. Environ. Contam. Toxicol.* **2013**, *4*, 377-381.
[82] Yu, Y.; Yang, D.; Wang, X.; Huang, N.; Zhang, X.; Zhang, D.; Fu, J., Factors influencing on the bioaccessibility of polybrominated diphenyl ethers in size-specific dust from air conditioner filters. *Chemosphere*, **2013**, *93*(10), 2603–2611.
[83] Takigami, H.; Suzuki, G.; Hirai, Y.; Ishikawa, Y.; Sunami, M.; Sakai, S.-i., Flame retardants in indoor dust and air of a hotel in Japan. *Environ. Int.*, **2009**, *35*(4), 688-693.
[84] Abdallah, M. A.-E.; Harrad, S.; Covaci, A., Hexabromocyclododecanes and tetrabromobisphenol-A in indoor air and dust in Birmingham, UK: Implications for human exposure. *Environ. Sci. Technol.*, **2008**, *42*(18), 6855-6861.
[85] Abdallah, M. A. E.; Harrad, S.; Ibarra, C.; Diamond, M.; Melymuk, L.; Robson, M.; Covaci, A., Hexabromocyclododecanes in indoor dust from Canada, the United Kingdom, and the United States. *Environ. Sci. Technol.*, **2008**, *42*(2), 459-464.
[86] Stapleton, H. M.; Allen, J. G.; Kelly, S. M.; Konstantinov, A.; Klosterhaus, S.; Watkins, D.; McClean, M. D.; Webster, T. F., Alternate and New Brominated Flame Retardants Detected in U.S. House Dust. *Environ. Sci. Technol.*, **2008**, *42*(18), 6910-6916.
[87] Abdallah, M. A. E.; Harrad, S., Personal exposure to HBCDs and its degradation products via ingestion of indoor dust. *Environ. Int.*, **2009**, *35*(6), 870-876.
[88] Harrad, S.; Abdallah, M. A. E.; Covaci, A., Causes of variability in concentrations and diastereomer patterns of hexabromocyclododecanes in indoor dust. *Environ. Int.*, **2009**, *35*(3), 573-579.

[89] Roosens, L.; Abdallah, M. A. E.; Harrad, S.; Neels, H.; Covaci, A., Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: Preliminary results. *Environ. Health Perspect.*, **2009**, *117*(11), 1707-1712.
[90] Roosens, L.; Cornelis, C.; D'Hollander, W.; Bervoets, L.; Reynders, H.; Van Campenhout, K.; Van Den Heuvel, R.; Neels, H.; Covaci, A., Exposure of the Flemish population to brominated flame retardants: Model and risk assessment. *Environ. Int.*, **2010**, *36*(4), 368-376.

Annex B

Supplementary data from subchapter 3.2: **Brominated, chlorinated and phosphate organic contaminants in house dust from Portugal.**

Table B1. Housing and sampling characteristics for each of the samples. The number of electronic devices refers to the total number of TVs, computers, stereos, VCRs and DVD players.

Sample	City	Construction year	Area (m^2)	Location	House type	No. of electronic devices
1	Aveiro	2006	130	urban	apartment	6
2	Aveiro	2004	215	agricultural	private house	7
3	Aveiro	2009	300	sub-urban	private house	7
4	Aveiro	2005	150	urban	apartment	8
5	Aveiro	2006	300	sub-urban	private house	7
6	Aveiro	2007	250	agricultural	private house	7
7	Aveiro	2002	90	urban	apartment	7
8	Aveiro	1961	100	urban	apartment	4
9	Aveiro	1860	150	urban	private house	4
10	Aveiro	2000	25	urban	apartment	3
11	Aveiro	2004	100	agricultural	apartment	6
12	Aveiro	1990	130	urban	apartment	10
13	Aveiro	2006	82	urban	apartment	5
14	Aveiro	**n.a.**	35	urban	apartment	5
15	Aveiro	1940	600	sub-urban	private house	11
16	Aveiro	**n.a.**	**n.a.**	**n.a.**	**n.a.**	**n.a.**
17	Aveiro	1995	260	agricultural	private house	7
18	Aveiro	1986	92.5	urban	apartment	6
19	Coimbra	1956	105	urban	private house	3
20	Coimbra	2001	100	urban	apartment	8
21	Coimbra	2000	145	sub-urban	private house	10
22	Coimbra	2007	120	urban	apartment	6
23	Coimbra	2000	240	sub-urban	private house	7
24	Coimbra	2002	200	urban	apartment	10
25	Coimbra	1990	190	urban	apartment	6

Sample	City	Construction year	Area (m^2)	Location	House type	No. of electronic devices
26	Coimbra	1989	100	urban	apartment	3
27	Coimbra	2001	100	urban	apartment	1
28	Coimbra	2007	70-80	urban	apartment	2

n.a.: information not available.

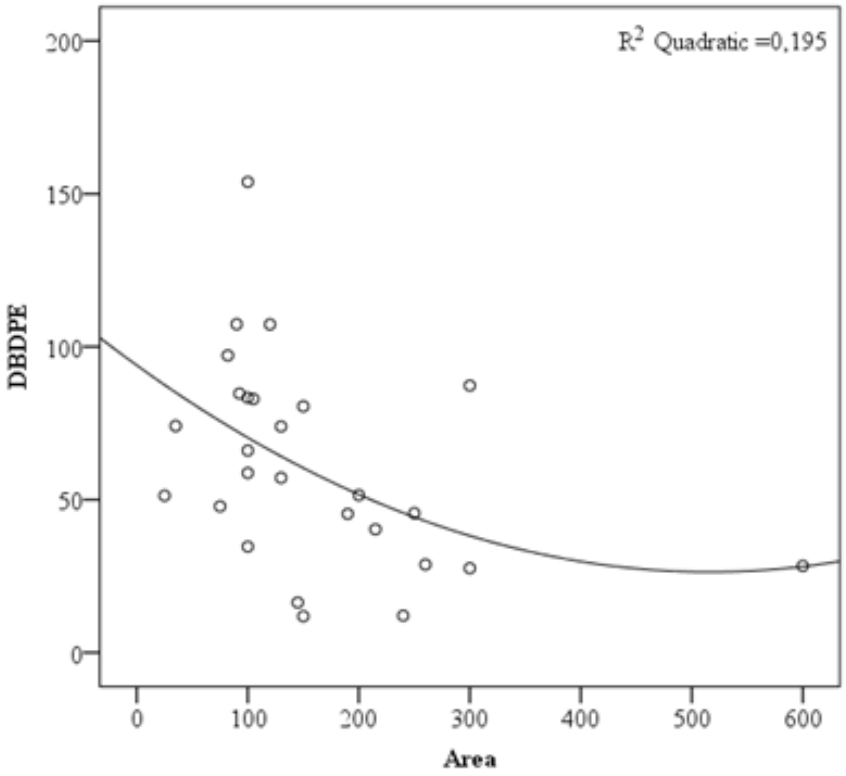

Figure B1: Relationship between the levels of DBDPE (ng g^{-1}) quantified in house dust samples and the houses' area (m^2).

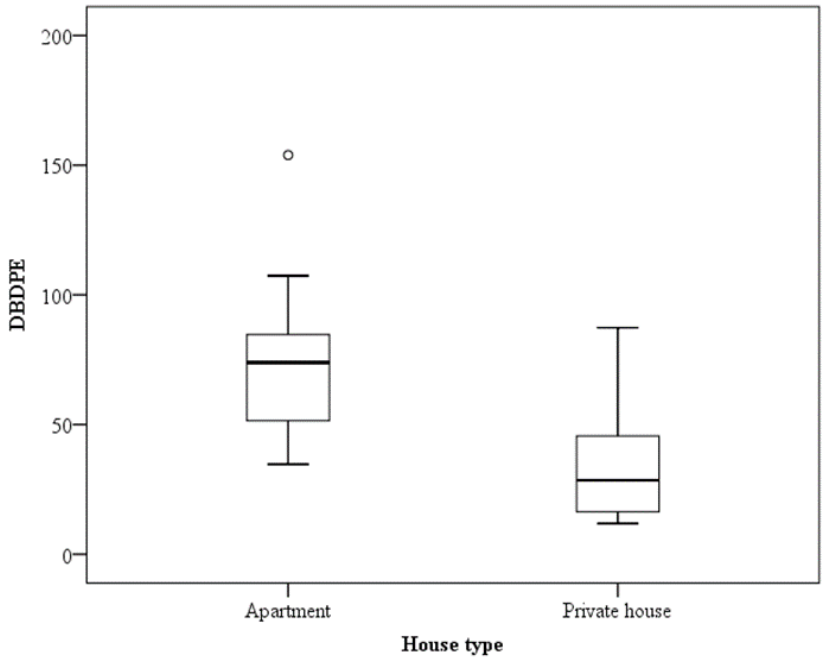

Figure B2: Boxplot summarizing the variation in the concentrations of DBDPE (ng g^{-1}) in apartments and private houses. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).

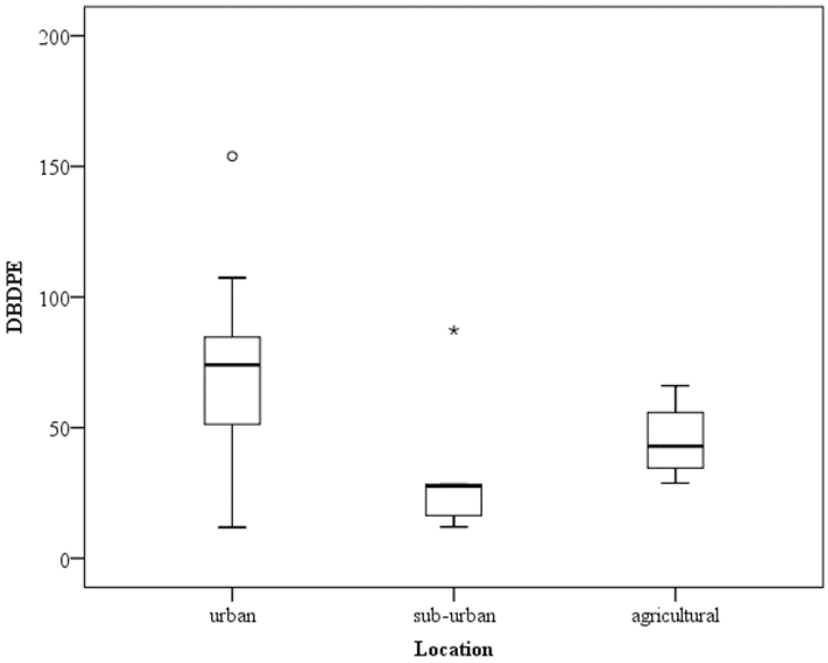

Figure B3: Boxplot summarizing the variation in the concentrations of DBDPE (ng g^{-1}) according to the location of the houses. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).

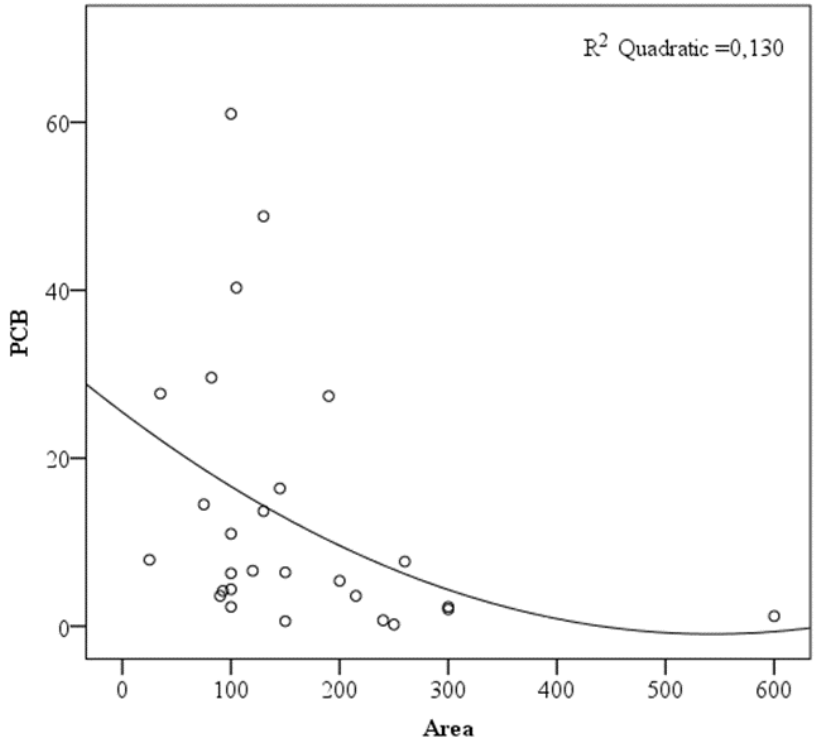

Figure B4: Relationship between the levels of total PCBs (ng g^{-1}) quantified in house dust samples and the houses' area (m^2).

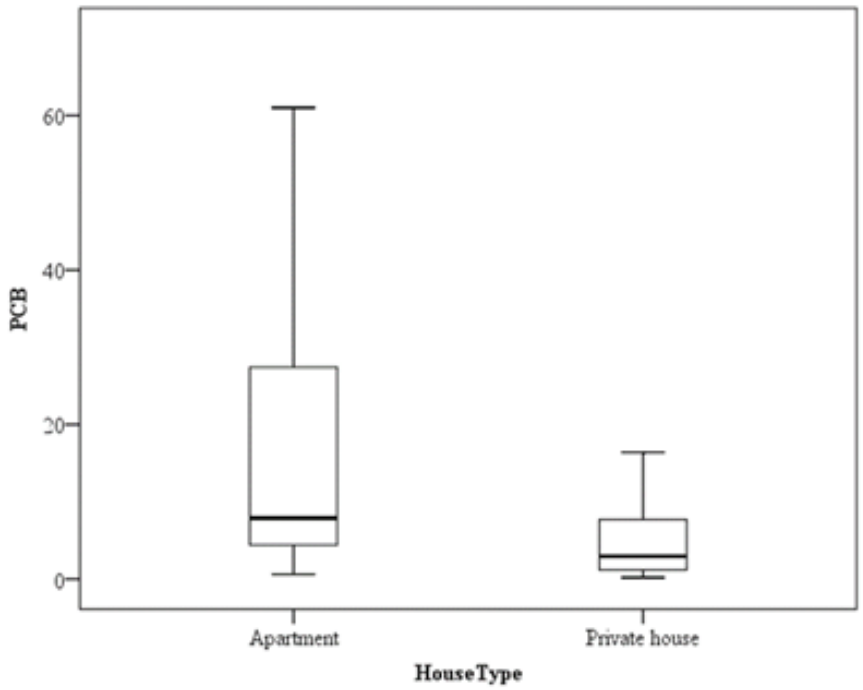

Figure B5: Boxplot summarizing the variation in the concentrations of PCBs (ng g^{-1}) according to the house type. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).

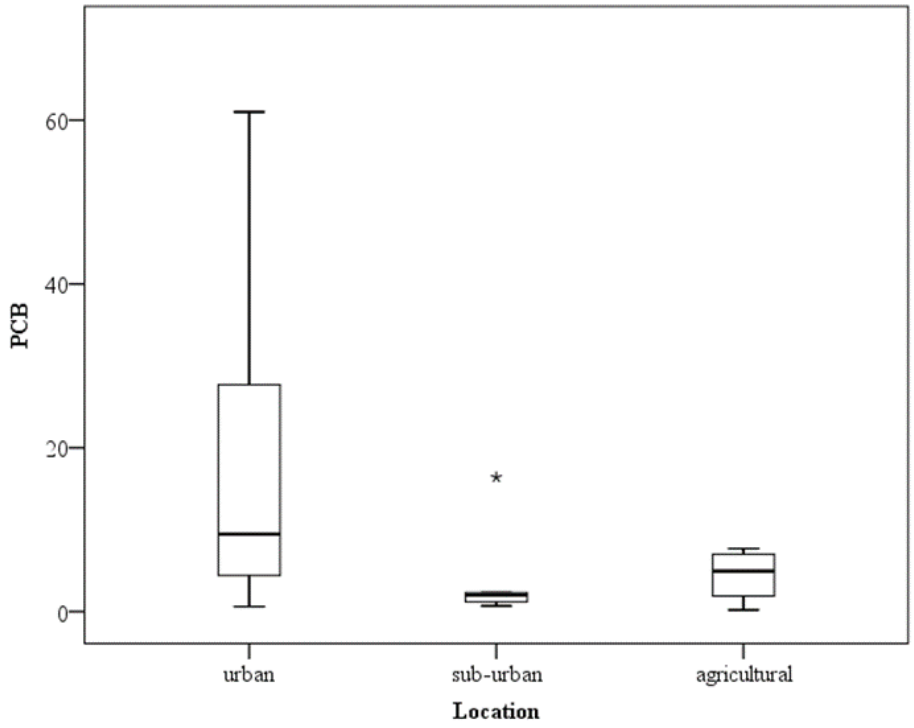

Figure B6: Boxplot summarizing the variation in the concentrations of PCBs (ng g^{-1}) according to the location of the houses. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).

Annex C

Supplementary data from subchapter 3.3: **Asthma and exposure to brominated flame retardants, polychlorinated biphenyls and organochlorine pesticides via house dust.**

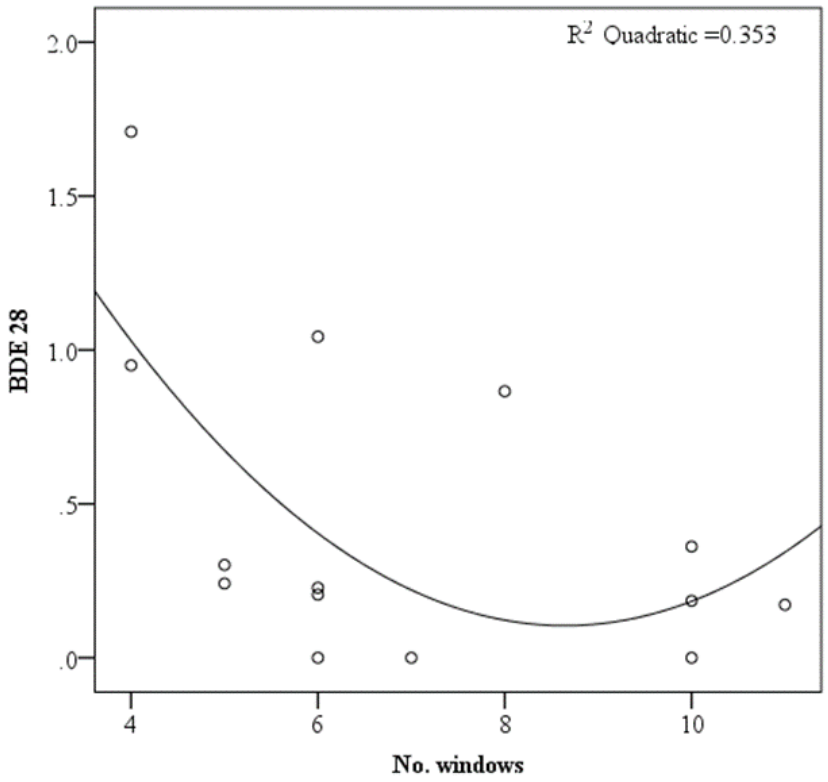

Figure C1: Relationship between the levels of BDE 28 (ng g^{-1}) quantified in house dust samples and the number of windows.

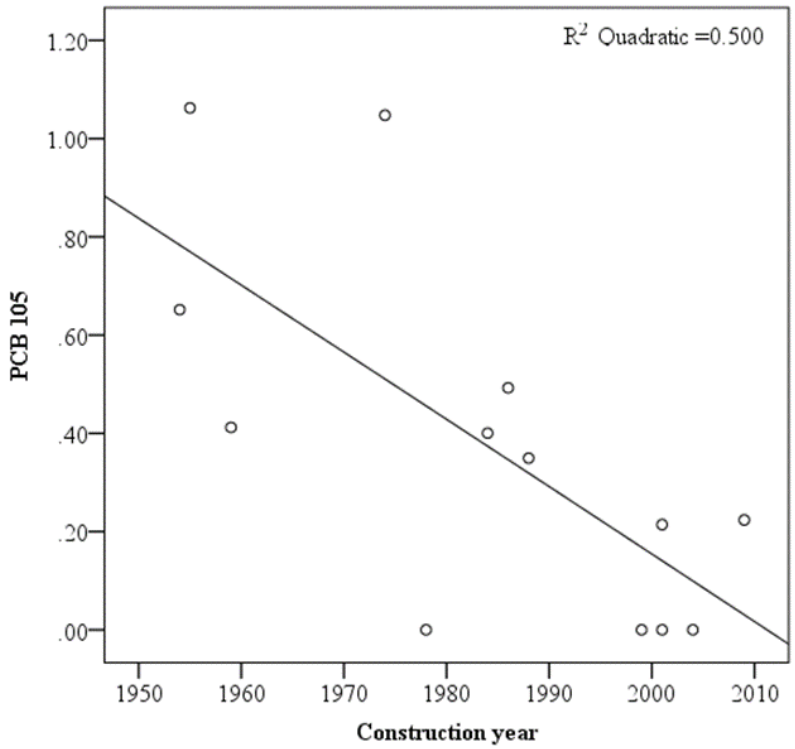

Figure C2: Relationship between the levels of PCB 105 (ng g^{-1}) quantified in house dust samples and the construction year.

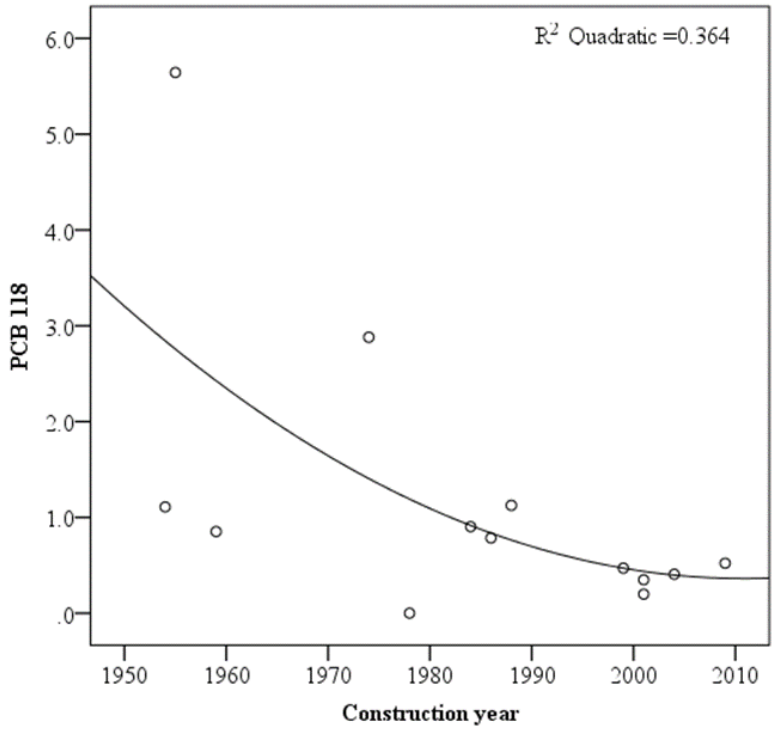

Figure C3: Relationship between the levels of PCB 118 (ng g^{-1}) quantified in house dust samples and the construction year.

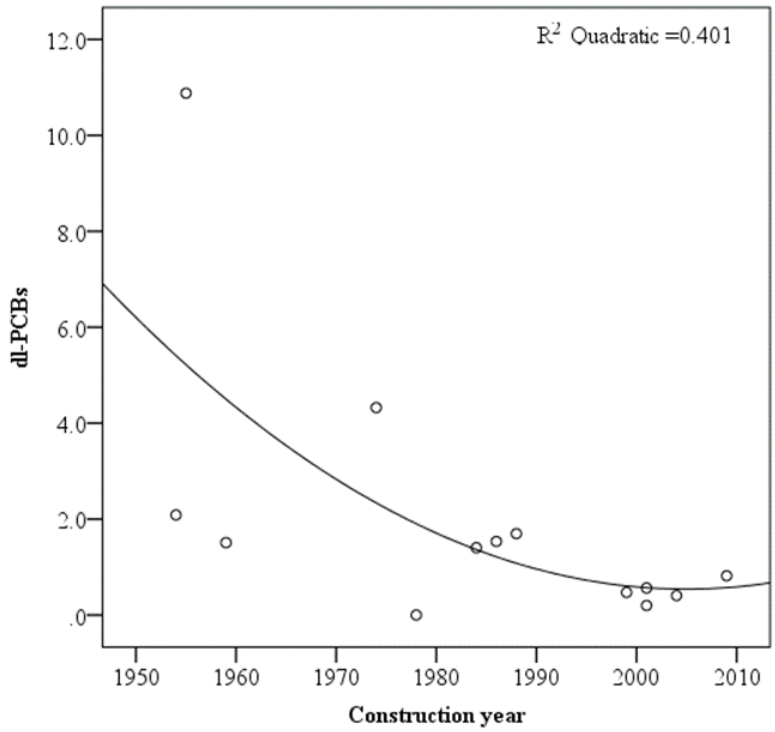

Figure C4: Relationship between the levels of dioxin-like PCBs (ng g^{-1}) quantified in house dust samples and the construction year.

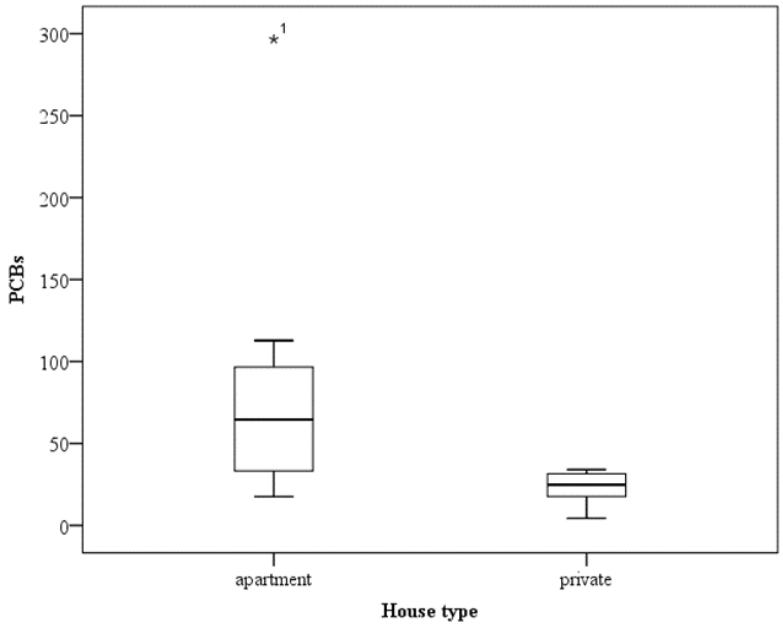

Figure C5: Boxplot summarizing the variation in the concentrations of PCBs (ng g^{-1}) according to the house type. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).

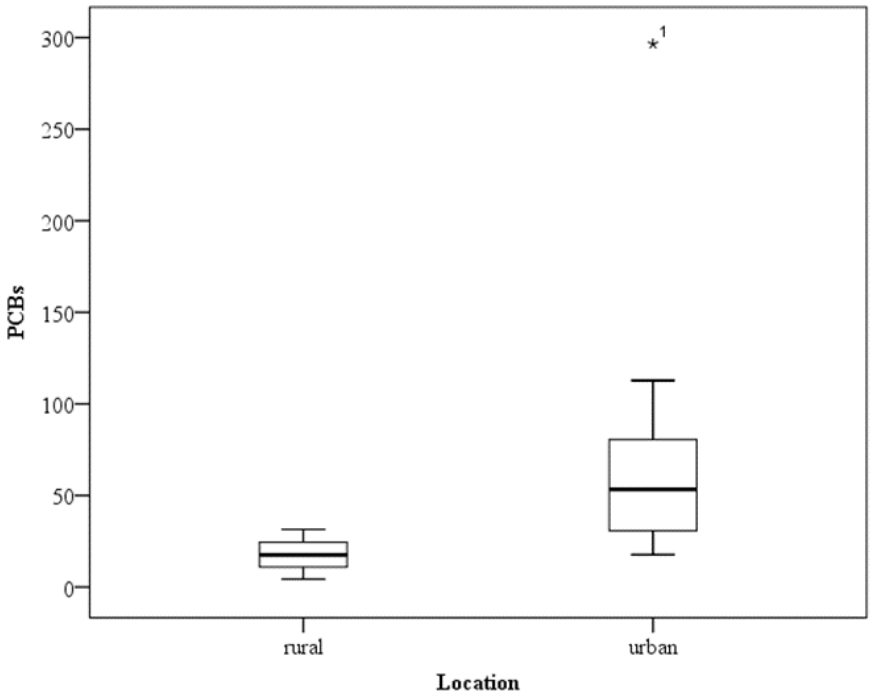

Figure C6: Boxplot summarizing the variation in the concentrations of PCBs (ng g^{-1}) according to the location. Outliers, maximum, minimum, median, and the 25th and 75th percentiles are presented (ng g^{-1}).